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NEWS

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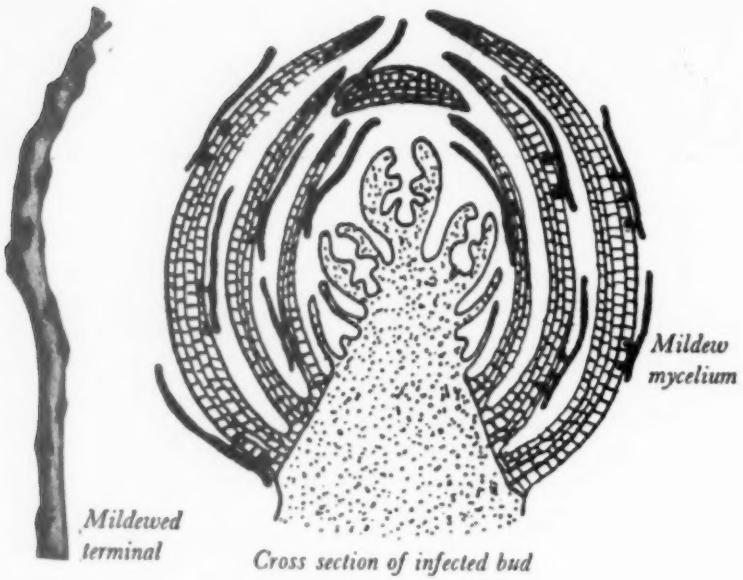

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SILICON CONTENT OF THE RICE PLANT AS A FACTOR INFLUENCING ITS RESISTANCE TO INFECTION BY THE BLAST FUNGUS, PIRICULARIA ORYZAE[1]

Richard J. Volk,[2] Robert P. Kahn, and Robert L. Weintraub

SUMMARY

A quantitative study has been made of the relationship between the silicon content of leaves of Caloro rice and their susceptibility to infection by *Piricularia oryzae* Cav. Silicon content and susceptibility are related inversely. The susceptibility of each leaf is greatest as it emerges; with advancing age the susceptibility diminishes until virtually complete resistance is attained. Both the silicon content and the degree of susceptibility of the leaf at any moment are related to the amount of silicate available to the roots. It is hypothesized that the silicon combines with 1 or more components of the cell wall to form a complex relatively resistant to attack by the extracellular enzymes of Piricularia, and so diminishes hyphal penetration into the leaf.

Silicon has been implicated repeatedly as a factor influencing the degree of susceptibility of rice to the blast disease caused by *Piricularia oryzae* Cav. Miyake and Adachi (17) noted a higher Si content in leaves of a resistant variety than in those of a susceptible variety. Adyanthaya and Rangaswami (1) and Venkatachalam (23) reported greater numbers of silicated epidermal cells in resistant than in susceptible varieties, but Hashioka (8) concluded that the degree of silicification is unimportant as a factor determining varietal differences in susceptibility. Several workers (4, 5, 13, 21, 22, 25) have claimed that susceptibility was diminished by increasing the supply of absorbable Si.

Evidence has been adduced that many other factors also are involved in determining susceptibility to blast (12, 19, 20). In some instances, the Si content or number of silicated cells has been shown to be greater under the conditions conducive to resistance (2, 3, 8, 9), suggesting that Si may be the factor of primary importance. In other cases, the role of Si was believed to be subordinate to or dependent upon other factors (11, 21, 22) such as the level of available nitrogen, magnesium, or water.

To establish the role of a single factor that, in all likelihood, is interacting with many others may be a very complex and difficult task. It is not surprising that current information as to the role of Si in resistance to blast is fragmentary and contradictory. The present study was undertaken in the hope that a better understanding of the problem might be gained by a quantitative investigation of the silicon-resistance relationship on an individual leaf basis.

METHODS.—*Culture of rice plants.*—Rice of the variety Caloro was used in all the experiments reported here. In 1 experiment, the plants were grown singly in ¼-gal. crocks on flooded Duffield silt loam. Nutrient culture was used in all the others, the plants being supported by Sponge-Rok, a heat-expanded aluminum silicate.

It was found that rice plants could be grown successfully on Hoagland solution No. 1 (10) if 15–20 ppm Fe as ferric ethylenediaminetetracetate (Fe EDTA) was substituted for the recommended source of Fe. The solutions (Table 1) were prepared with distilled water containing less than 1 ppm Si. Where silicate was to be included, sulfuric acid was first added to the basal nutrient solution in such amount as to result in a hydrogen-ion concentration between pH 5 and pH 6 after addition of the sodium silicate. In order to eliminate the sodium content as another variable, the final concentration of sodium sulfate was adjusted to the same level in each solution. All the nutrient solutions were adjusted to pH 5.2 and Fe EDTA added just prior to use. The nutrient solutions were replaced weekly.

Inoculation and evaluation.—Leaves were inoculated by exposing them to a uniform spore deposition in a modified form of the settling tower described by Bell et al (6). Two isolates of *Piricularia oryzae* were used in the various tests: No. 603, Race 2 (14, 15), and No. 640, representing a hitherto undescribed race. The inoculation apparatus consisted of a 6 × 6 × 3-ft. wooden base cabinet housing a turntable and supporting a sheet metal cylinder 5 ft. high and 2½ ft. in diameter. The turntable, which was 5 ft. in diameter, bore at the periphery 8 tapered slots to accommodate 8 pots of plants in a horizontal position. The plants were positioned so that the leaf blades were oriented under the center of the cylinder. A predetermined quantity of spores was discharged upward into the tower by a CO_2 pistol provided with an adapter that directed the blast vertically through a small hole in a baffle plate between the turntable and cylinder. The movable baffle was kept in position for 1 minute after discharge to intercept any spore clumps that might be present. The plate was then removed and the leaves exposed to the spore shower for 11 minutes, after which the test plants were transferred to dew cabinets (16) at 80°F. After 16–18 hours, the plants were transferred to a greenhouse for an additional 4–6 days prior to the counting of the lesions on the laminae.

The absolute number of lesions per unit area was determined by several factors, including the density of the spore discharge, which varied from experiment to experiment. Consequently, comparisons of lesion

[1] Accepted for publication October 21, 1957.

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counts should not be made among experiments but only among the various treatments within each individual experiment.

Silicon determination.—Rice-leaf samples to be analyzed for total silicon were dried at 70°C, weighed to the nearest 0.1 mg, and ground in a Wiley mill to pass a 20-mesh screen. In order to avoid silicon contamination during grinding, an aluminum plate was substituted for the glass plate ordinarily used to cover the blades of the mill. After being mixed thoroughly, the ground samples were redried at 70°C and stored in a desiccator prior to analysis. When an individual leaf weighing less than 50 mg was to be analyzed for silicon, the entire leaf was cut up—not ground—and used for analysis.

The method used for determination of silicon was a modification of the molybdenum blue spectrophotometric procedure described by Mullin and Riley (18) for water analysis. For maximal precision, the analytical sample should contain 25–2500 μg Si. A detailed description of the procedure will be published separately.

Nitrogen determination.—Organic nitrogen was determined by the Kjeldahl micromethod.

EXPERIMENTAL RESULTS.—*Growth of rice plant.*—Growth curves for the successive leaves of Caloro rice plants cultured in soil in the greenhouse are shown in Figure 1. In this and following figures, the leaves are designated by Roman numerals starting with I for the first seedling leaf.

Inasmuch as there was a very close correlation between length and dry weight of the leaves ($r = 0.95$), a nearly identical set of curves would result if weights rather than lengths were plotted as ordinate.

Susceptibility to blast in relation to leaf position and age.—Figure 2 shows that as a rice leaf ages its resistance increases progressively, even after growth has ceased. Thus when a leaf first becomes visible, its resistance is minimal or, conversely, its susceptibility is maximal. As indicated by the over-all resistance pattern of leaves as they age (Fig. 3), the maximal susceptibility of each successive leaf is less than that of the preceding ones. It is apparent also that each successive leaf remains susceptible for a shorter period of time after its appearance than did the preceding leaves. These "positional" effects are presumed to be due to the progressively greater intervals that the successive leaves remain within the sheath bundle prior to their emergence. In other words, the age of the leaf at emergence, counting from the initiation of its growth from the primordium, is progressively greater in the successive leaves, so that 2 leaves of different position, though equally recently emerged, may actually differ greatly in developmental or physiological age.

If the plant is subjected to a single inoculation so that all the emerged leaves are subject to infection at the same time, the susceptibility pattern will be as shown in Figure 4. The interaction between leaf age and leaf position is such that at any moment the plant as a whole will exhibit a gradient of resistance increasing from the younger upper leaves toward the older basal leaves. In older plants, however, the gradient tends to disappear, and the plant as a whole becomes resistant because of the decreased maximal susceptibility and duration of susceptibility (Fig. 3).

Absorption of silicon and relation to growth.—The rice leaf can accumulate Si in very large amount. Figure 5 illustrates the results of an experiment in which plants were cultured in nutrient solution with or without added silicate. Accumulation by the leaf appears to continue indefinitely if the root is provided with absorbable Si. There was no consistent evidence that either the growth rate or the final size of the leaf was influenced by addition of silicate to the medium.

The amount of silicon accumulated by a leaf varies directly with the concentration of supplied silicate up to about 60 ppm (Fig. 6). Concentrations of Si in the leaf as high as 7 per cent of the total dry weight were found in high-silicate cultures with complete nutrient; even greater concentrations occurred under conditions of nitrogen deficiency.

The Si status of the leaf is closely responsive to the external silicate supply. For example, in 1 experiment the fifth leaf blade of plants on a low-Si nutrient (< 3 ppm) contained 0.77 per cent Si 3–4 days after attaining maximal length. Some of the plants were then transferred to nutrient containing approximately 115 ppm Si. The leaves of these plants absorbed Si rapidly and their content rose in 5 days to 5.14 per cent, whereas that of the low-Si plants was virtually unchanged (0.82 per cent).

The foregoing results indicate that the Si content of various portions of the "normal" rice plant may vary within wide limits, depending on the availability of absorbable Si and of other elements in the root

TABLE 1.—*Composition of nutrient solutions used for culturing rice in the greenhouse*

Stock solution		Ml stock solution per liter nutrient solution	
Component	Concentration	A [a]	B [b]
KH_2PO_4	1.0 M	1	
K_2HPO_4	1.0 M		1
K_2SO_4	0.5 M	5	3
$Ca(NO_3)_2$	1.0 M	5	
$CaSO_4$			(0.34 g/liter)
$MgSO_4$	1.0 M	2	2
Trace elements [c]		1	1
FeEDTA [d]	5000 ppm	4	4
NH_4NO_3	1.0 M		0–7.5
H_2SO_4	25% (v/v)	0–0.88 [e]	0–0.44 [e]
Na_2SiO_3	1.0 M	0–4	0–2
Na_2SO_4	1.0 M	0–4 [f]	0–2 [f]

[a] A solution used in experiments in which Si supply was only variable.

[b] B solution used in experiments in which both Si and N supply were varied.

[c] Stock trace element solution contained 2.86g H_3BO_3, 1.81g $MnCl_2 \cdot 4H_2O$, 0.22g $ZnSO_4 \cdot 7H_2O$, 0.08g $CuSO_4 \cdot 5H_2O$, and 0.02g $H_2MoO_4 \cdot H_2O$ in 1 liter water.

[d] Ferric ethylenediaminetetracetate.

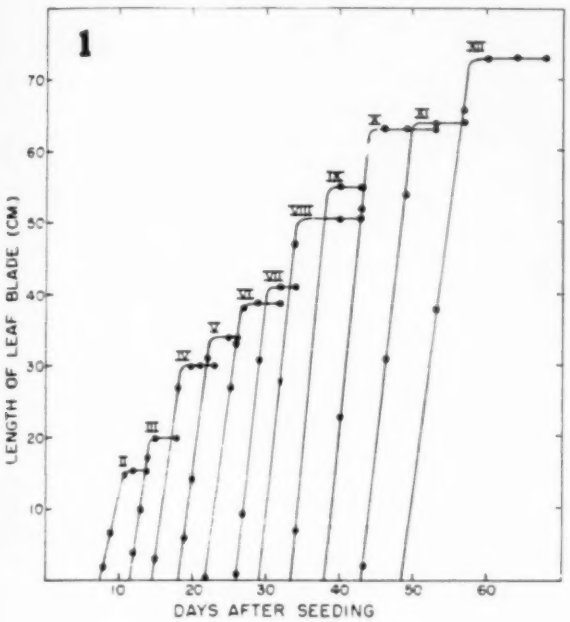
[e] 0.22 ml for each ml of 1 M Na_2SiO_3 added.

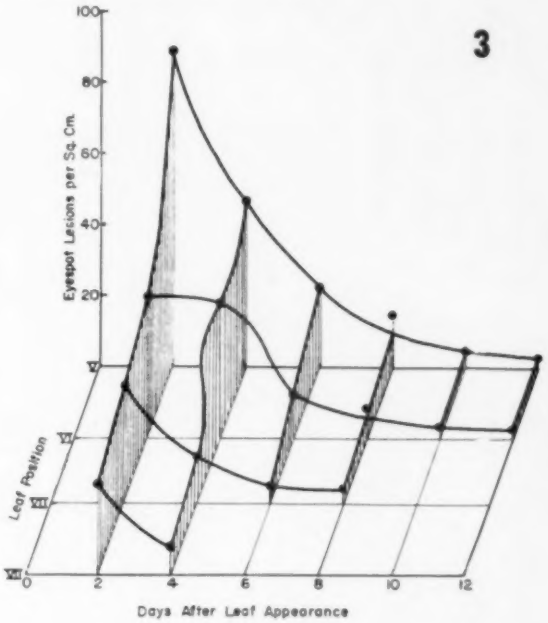
[f] Four ml minus the number of ml of 1 M Na_2SiO_3 added.

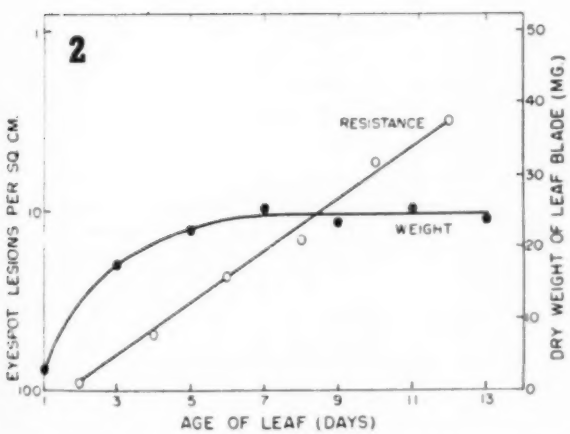
environment.

Relation of silicon to blast.—The resistance to blast of plants cultured in nutrient solution can be increased greatly by addition of silicate. In young leaves, resistance appears to be related directly to the concentration of silicate in the nutrient solution (Fig. 6).

As the leaf ages, its resistance increases rapidly and becomes virtually complete whether the silicate supply is large or small (Fig. 7). Presumably the resistance depends upon the Si content of the leaf up to a quite definite level; once this level is reached, resistance is practically maximal and obviously cannot be further increased by additional absorption of Si. This situation is illustrated by Figure 8.







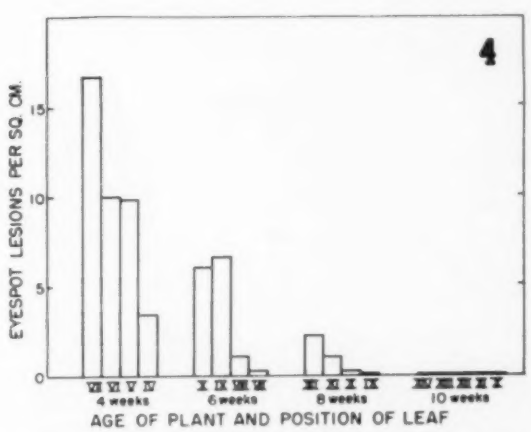
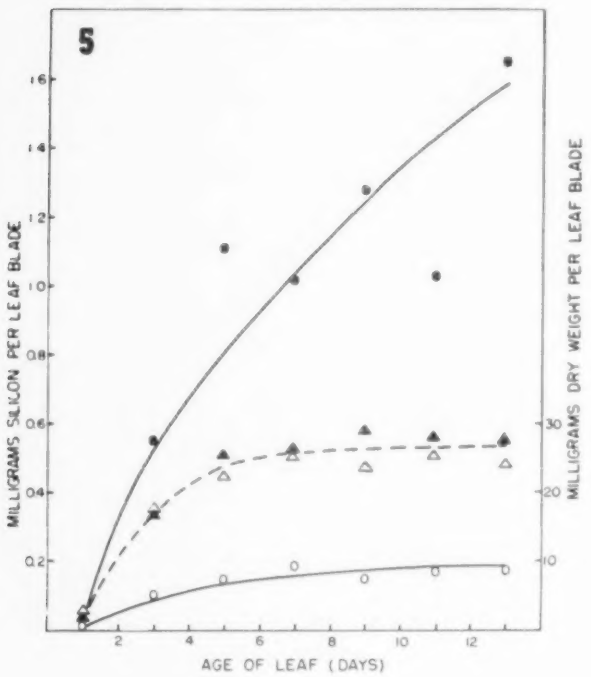
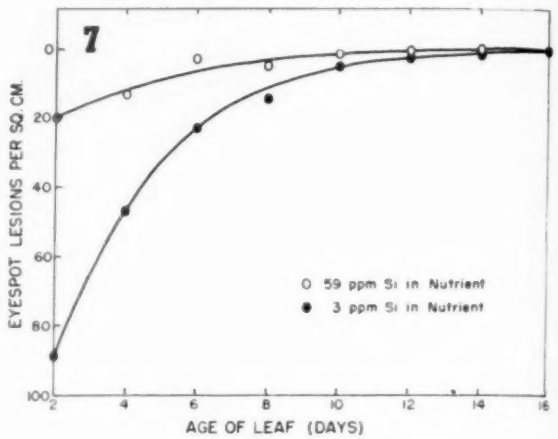
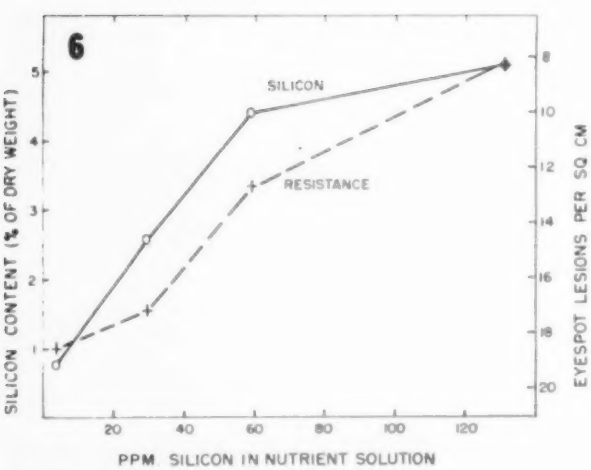


FIG. 1-4. Fig. 1. Growth curves of successive leaves of Caloro rice cultured in flooded soil in the greenhouse. First leaf designated I, second leaf II, etc.—Fig. 2. Growth curve and change in blast resistance of rice leaf with age. Leaf V, solution culture, Piricularia strain 640.—Fig. 3. Relation of blast susceptibility to position and age of rice leaf.—Fig. 4. Pattern of susceptibility of various leaves of rice plants at several ages from planting. Flooded soil culture, Piricularia strain 603.

Relation of nitrogen to blast.—There is general agreement in the literature that susceptibility to blast is favored by nitrogen fertilization of the plant. This is shown also in Table 2, which summarizes results from an experiment in which nitrogen was omitted from the "minus N" nutrient solutions for 7 days prior to inoculation. Two plants were grown in each pot; 1 was harvested for analysis just prior to inoculation of the other. It is apparent that the nitrogen content of the uppermost leaves had not been affected by this relatively short period of nitrogen deletion; there was no significant treatment effect on leaf weight through-







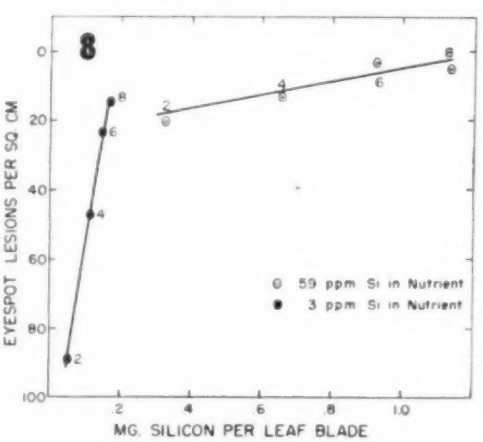


FIG. 5-8. Fig. 5. Growth and silicon accumulation by rice leaves as influenced by silicate supply. Leaf V, solution culture. Continuous curve and circles indicate Si content; broken curve and triangles represent leaf weight. Open points: 3 ppm Si in solution; solid points: 59 ppm Si in solution.—Fig. 6. Silicon content and blast resistance as influenced by silicate supply. Leaf V, age 8 days, Piricularia strain 640.—Fig. 7. Variation of blast resistance with age of leaf at 2 levels of silicate supply. Leaf V, Piricularia strain 640.—Fig. 8. Resistance to blast of young rice leaves as related to silicon content of leaf. Leaf V, Piricularia strain 640. The numbers at each point designate leaf age in days from its emergence from the sheath.

out the course of the experiment. Withholding of nitrogen, however, did permit the absorption of a significantly larger amount of Si when the latter was supplied in abundance. The number of lesions/cm² correlated with the Si content but not with the N content.

Another indication of the effect of nitrogen supply on Si uptake was obtained from a field experiment conducted by Dr. E. C. Tullis and co-workers. Zenith rice was grown in soil at various levels of N fertilization. As shown in Table 3, the Si content, on either an absolute or a percentage basis, was markedly greater in the leaves of the minus-N plants. Hence, there is a strong suggestion that the often-observed influence of N fertility on blast may be related directly to the amount of Si absorbed.

DISCUSSION.—Inasmuch as penetration of the rice leaf by *Piricularia oryzae* is ordinarily through the cuticle rather than via the stomates (24), it may be assumed that during the process of hyphal penetration there is an enzymatic degradation of cell-wall components such as cellulose, lignin, and pectic substances. It seems not unlikely that chemical modification of such structural constitutents would result in diminished susceptibility to enzymatic attack.

Engel (7) has demonstrated that the Si in oat straw is present in at least 3 forms: 1) an insoluble polymerized silicic acid; 2) a soluble inorganic Si fraction; and 3) an organic-Si complex, consisting of a rather stable, esterified Si-galactose unit loosely associated with other organic constituents and phosphorus. Of special significance is the fact that the molar ratio of SiO_2 to galactose changes from 1:1 in immature oat straw to 2:1 in mature straw, whence Engel suggests the existence of a series of SiO_2-sugar components.

If it is assumed that Si is incorporated into the organic structural components of the rice leaf, a working hypothesis may be propounded to explain the relationship between Si and resistance to blast. Briefly, it postulates the production in the leaf of 1 or more components, X, that by union with inorganic Si may form complexes, X-Si_m, either before or after being incorporated into the cell wall. These Si-containing constituents of the cell wall are assumed to be relatively resistant to enzymatic attack by the penetrating hyphae, thereby limiting the infection at the penetration stage.

According to the hypothesis, X is produced and incorporated into the wall during the period of active cell growth and wall formation. Its production may be presumed to be influenced by the nitrogen status of the leaf; an excess of nitrogen might limit the synthesis of X by shunting carbohydrate precursors toward the synthesis of nitrogenous constituents as well as by diminishing the uptake of Si.

The rate or amount of X-Si_m formed would be expected to depend also upon the concentration of silicate in the cell during the growth phase.

This hypothesis appears capable of explaining all the factors shown experimentally in this study to influence susceptibility—supply of silicate and nitrogen, age and position of leaf. 1) The decreased susceptibility occasioned by supplying silicate is due to an increase in the amount of an organo-silicon constituent of the epidermal cell wall that renders it less easily attacked by the fungal enzymes. 2) The increased susceptibility that accompanies high nitrogen supply is due to a decrease in the amount of the postulated organo-silicon constituent, presumably because of interference by the nitrogen either with Si uptake or with the formation of the precursor of the complex. 3) Under the usual cultural conditions, the relatively small amount of Si required to render the cell resistant to attack is available and becomes incorporated into the wall early in leaf ontogeny; this explains the observed rapid increase in resistance as a leaf ages. 4) Because the Si combines with the wall component principally or exclusively during the early stages of leaf growth, this union will be the more nearly completed the longer the period of leaf development within the sheath bundle prior to its emergence; hence the leaves of higher position exhibit less susceptibility at the time of emergence.

TABLE 2.—*Susceptibility to blast of the top leaf (leaf IV) of 41-day-old Caloro rice plants as influenced by the supply of nitrogen and silicon*

Nutrient	Eyespot lesions per sq. cm. [a]	Analysis of leaves at inoculation date	
		% N	% Si
Minus N, Minus Si	5.0	5.7	0.48
Minus N, Plus Si	2.3	5.3 [b]	2.12
Plus N, Minus Si	7.4	5.5	0.35
Plus N, Plus Si	4.2	5.5	1.32

[a] Averages of 12 replications.
[b] Estimated value.

TABLE 3.—*Silicon content of the uppermost leaves of 35-day-old, field-grown Zenith rice receiving various levels of nitrogen fertilizer*

Nitrogen fertilizer level (lb. per acre)	Silicon content [a]	
	mg per leaf	Percentage of dry weight
0	0.40	0.78
100	0.19	0.44
300	0.20	0.42

[a] Each figure is the mean from duplicate leaf samples.

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FACTORS AFFECTING DEVELOPMENT OF BACTERIAL STEM ROT OF PELARGONIUM[1]

A. Kivilaan and R. P. Scheffer

SUMMARY

A high percentage of commercially available pelargonium stocks carry *Xanthomonas pelargonii* in a latent form. Twenty-six per cent of 600 plants of 6 varieties, kept under conditions favoring disease development but precluding disease spread, developed stem rot within 3 months. *X. pelargonii* did not attack plants outside the geranium family. Isolates of the causal organism were found to differ qualitatively and quantitatively in effects on host plants. The pathogen was isolated from vessels of inoculated plants but caused stem rot only when parenchyma was invaded. Root invasion was demonstrated. Disease was favored by high temperatures: symptoms developed slowly at 10° C night temperature, developed faster at 21°, and rapidly killed plants at 27°. Mineral nutrient levels below and above optimum for growth enhanced disease development, as did high N, high P, and low Ca. Symptoms were inhibited by high K, low P, high Ca, and balanced nutrient solution at optimum concentrations for growth.

Pelargoniums once were comparatively free of destructive diseases, but in recent years serious losses have resulted from bacterial leaf spot and stem rot (blight), caused by *Xanthomonas pelargonii* (Brown) Starr et Burk. These diseases have been known for many years and are widely distributed. Today the pathogen can be found in most commercial stocks, and the diseases have become limiting factors in production.

Very little is known of the effects of environmental factors on blight development, although other aspects of the disease have been studied thoroughly. Brown (2) considered the disease of little consequence unless plants were weakened by rapid growth, high temperature, or high humidity. Munnecke obtained information on the host range, varietal susceptibility, latent infections in cuttings, and dissemination (7) and on survival of the organism in soil (8). In general, his results agreed with and extended the findings of Hellmers (4).

The original habitat of pelargonium was the Great Karroo region of South Africa. Jurassic soil with high potassium, moderately high altitude with cool nights and hot days, and low rainfall are important characteristics of the Great Karroo environment (1). Such factors should be considered in a study of environmental effects on diseases. There are many cultivated varieties of pelargonium (*Pelargonium hortorum* Bailey, *P. zonale* Ait., *P. peltatum* Ait., and *P. domesticum* Bailey), but all seem to do best in an environment resembling that of the original habitat. An attempt was made in the present work to evaluate the role of certain environmental, host, and pathogen factors affecting disease development. The primary aim of the project was to learn the optimum conditions for disease development so that disease-free propagating stocks could be selected. Temperature, mineral nutrition, and latent infections received special emphasis. A preliminary report has been published (5).

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MATERIALS AND METHODS.—*Pelargonium hortorum* var. Ricard was used in most experiments, since it is moderately susceptible to bacterial blight. Cuttings of Better Times and Radio Red varieties and seedlings of *P. hortorum* were used in some experiments. In order to eliminate plants with latent bacterial infections, rooted cuttings in 3-in. pots were kept for 3 weeks at 27° C night temperature. During the preexperimental period, all plants were watered daily with distilled water and fed weekly with a nutrient solution.

Xanthomonas pelargonii was isolated from infected pelargonium stems. Stock cultures were kept at 4°–6° C on autoclaved potato plugs from which fresh cultures for inoculum were started on potato-dextrose broth. Isolates most frequently used were kept also in stock plants for reisolations when needed. Inoculum was prepared from cultures grown for 48 hours on potato-dextrose broth under constant shaking. Pathogenicity of cultures was checked at intervals.

Stems of test plants were inoculated by puncturing the stem with a needle and inserting a small cotton plug dipped in bacterial suspension. This method was selected after preliminary experiments showed that it was superior to several other methods, including injection through hollow glass microneedles. In some cases, leaves were inoculated by applying bacterial suspensions with an atomizer, after which plants were kept in a fog chamber for 48 hours.

Gallegly and Walker's solutions (3) were used to determine the effects of nutrient concentration on disease development. The basal (1H) solution was reduced to one-tenth (0.1H) and to one-half (0.5H) and increased 2 (2H) and 3 times (3H). High-nitrogen, high-phosphorus, and high-potassium solutions were prepared by increasing the amounts of the respective ions contained in the basal solution by 3 times. Solutions for low nitrogen, phosphorus, and potassium were prepared by decreasing the amounts of these ions contained in basal solution to one-third of normal. All other constituents were kept at 1H level. Osmotic pressures of the solutions were kept on the same level by adding calculated amounts of NaCl. All solutions contained the same amount of microelements (A–Z

solution) (3). Basal (1H) solution was considered as the high-calcium level. For the medium level, calcium was reduced to one-fifth and for the low level to one-seventeenth the calcium level of the normal solution.

Sodium sequestrenate, which often is used as a source of iron, was found to be very toxic to pelargoniums. For this reason $FeCl_3 \cdot 6H_2O$ was used as the iron source.

For nutrition experiments, cuttings (var. Ricard) were selected for uniformity and were grown under uniform conditions preceding inoculation. Roots were washed and the plants were set in clean white silica sand (grade No. 4) in 8-in. varnished clay pots. Each pot was provided with a siphon for drainage in such a way that about 750 ml was left undrained in the bottom (3). Four rooted cuttings were planted per pot. After 4 weeks, during which the plants were fed daily with 0.5H solution and the sand flushed weekly with distilled water, plants were once more selected for uniformity. Scions were taken from each plant for virus indexing since certain virus diseases are known to affect bacterial blight development (6). After being fed separately for 30 days at the various nutrient levels, all plants were stem inoculated with a virulent isolate. In all nutrient experiments, plants were fed daily and watered once a week with distilled water to minimize possible salt accumulation in pots.

The experimental design used was a randomized block with 3–7 replications, depending on the number of treatments. Each treatment thus contained 9–15 pots, with 36–60 plants. Disease readings were taken 50 and 90 days after inoculation; only the 50-day readings were analyzed statistically. These readings were based on the following classification: 0—healthy; 10—slight but positive symptoms; 20—slight darkening of stem, occasional partial wilting of leaves; 40—blackening of stem, shedding of lower leaves; 60—black stem rot with occasional tip necrosis; 80—severe stem rot; 100—dead.

HOST FACTORS.—*Tissues affected and movement of bacteria in plants.*—The possibility of root infection from infested soil is important, and root inoculations were attempted. Seedlings of *P. hortorum* were grown in 3-in. pots so that the main root penetrated through the hole of the pot. Twenty-five such plants were inoculated by dipping the free roots in bacterial suspensions in flasks below the pots. Roots of 25 control plants were dipped in water. Seventeen plants developed positive stem rot symptoms as a result of root inoculation.

There is some question as to whether *X. pelargonii* is a systemic pathogen or a parenchyma invader; leaf spot is believed to involve strictly parenchyma invasion (4). Ninety Ricard cuttings (average weight 15 g each) from stock plants free of stem rot were allowed to take up bacterial suspensions (100,000 cells per ml) of isolate 182 by transpirational pull for 24 hours, after which they were placed in sterile sand in 3-in. pots. Another group of 45 cuttings was kept as non-inoculated controls. Isolations from treated cuttings immediately after inoculation showed that bacteria were in vessels. A week after treatment, half the inoculated cuttings were perforated at the 2.5- and 5-cm levels above the soil line. Five weeks later, 38 inoculated but unwounded (except at the cut end) plants showed basal stem rot but no rot at other places. Of the 45 wounded plants, 35 had basal stem rot, 21 had rot at the 2.5-cm wound, and 8 had rot at the 5.0-cm wound. The pathogen was isolated from the vessels of all inoculated cuttings. Microscopic examinations of rotting stems showed deterioration of parenchyma in both pith and cortex. All 45 check plants formed roots and were free of disease. The results indicate that wounds may be needed for rot to start, even though the pathogen is in the vessels. Rot frequently starts near stipules and at leaf scars, which seems to support this observation.

Two groups of 25 cuttings each (var. Ricard) were allowed to take up bacterial suspensions for 24 hours. Basal ends of cuttings in 1 group were washed, surface sterilized with mercuric chloride (1:1000), and placed in 3-in. pots containing sterilized soil. The other group of 25 cuttings served as inoculated but otherwise untreated controls. After 5 weeks, 7 cuttings from the group treated with mercuric chloride had typical basal stem rot and 18 were normal, whereas 16 plants from the control group had stem rot and 9 were normal. The pathogen was, however, present in the symptomless plants, as determined by isolations. These tests further support the suggestion that even though *X. pelargonii* is present in vessels, it must be in contact with parenchyma before external symptoms develop.

The ability of vessels to conduct bacteria was determined by isolations. Cuttings from plants (var. Ricard) about 15 cm long and known to be free of stem rot were allowed to take up bacterial suspensions by transpirational pull. After the plants had taken up inoculum for 6, 12, and 24 hours, isolations were attempted from stems, petioles, and leaves. In most cases, bacteria were present in the stem vessels after the cuttings were left for 6 hours in bacterial suspension. In a few cases, the pathogen was found in petioles of the lowest leaves of cuttings left in suspension for 24 hours, but it was never found in leaf blades.

In another test, the movement of bacteria was traced

TABLE 1.—*Acidity of expressed sap of pelargoniums*

Species	Tissues and plant parts used	pH
Pelargonium hortorum [a]	Diseased stem	8.0
	Healthy plants	
	Leaf blade	3.9
	Top stem	4.8
	Middle stem	5.0
	Bottom of stem	5.6
	Center of stem	
	Pith	4.8
	Xylem	5.1
	Cortex	5.3
P. melissimum	Healthy leaves	3.3

[a] The seedlings used were 18 months old.

in plants (var. Ricard) inoculated by inserting the pathogen into the stems by means of cotton plugs. At 50 and 100 days after inoculation, the stems above the point of inoculation were cut in sections and isolations were made. The pathogen moved as much as 15 cm in 50 days and up to 25 cm in 100 days. The pathogen moved independently of symptom expression and was found well above rot, indicating a true systemic characteristic. Petioles seldom were invaded.

There is some difference of opinion regarding ability of the pathogen to become systemic from leafspots (4, 7). Eighty-seven attempts were made to isolate the pathogen from green leaf tissue surrounding bacterial leaf spots, and 123 isolations were attempted from petioles of heavily spotted leaves. Results were negative in all cases, an indication that *X. pelargonii* does not become systemic from leaf spots.

Some pH factors.—The lower pH limit for growth of *X. pelargonii* in potato-dextrose broth was found to be pH 5.6, whereas the upper limit was pH 8.4, which agrees closely with previous findings (2). Although the bacterium was sensitive to acid, it still grew in pelargonium plants with a sap pH well below the lower limit for growth in culture (Table 1). The acidity of plant parts varied somewhat: sap from leaves was always more acid than sap from stems, lower parts of stems were less acid than top parts of stems, and pith sap had a lower pH than did cortex sap. This variation may or may not explain the relative susceptibility of various plant parts and tissues, since sap from diseased portions was alkaline (Table 1). Presumably, this was the result of infection.

Prevalence of latent infections in commercial stock.—One hundred plants each of 6 common varieties were obtained from commercial sources in Michigan. Initially, all plants appeared to be healthy. They were potted in individual pots, well spaced on a steamed bench, and were carefully watered to prevent wetting of foliage and spread of pathogens. Night temperature in the greenhouse was maintained at 22° C. Plants that developed possible symptoms were separated from the group and isolations were made. Within 3 months, the following percentages of plants of the 6 varieties developed blight symptoms and in each case the pathogen was isolated: Radio Red, 77; Ricard, 23; Olympic Red, 9; Irvington Beauty, 4; Enchantress Supreme, 32; and Fiat, 13. The number of latently infected plants in a stock is an important factor in subsequent disease development and spread.

Host range.—The main purpose of these experiments was to find other hosts that might harbor the organism as a source of inoculum for pelargonium. Hellmers (4), Munnecke (7), and Starr et al (11) have previously determined the hosts in the geranium family and found susceptibility of various degrees in the following species: *Pelargonium hortorum* Bailey, *P. domesticum* Bailey, *P. peltatum* Ait., *Geranium maculatum* L., *G. sanguineum* L., *G. pratense* L., *G. sylvaticum* L., and *G. yedoense* Franch. et Sav.

Plants of 93 species belonging to 40 families were inoculated and reisolations were attempted after 1–2 months. Inoculations of at least 3 plants of each species were made in greenhouse or in plants grown out-of-doors in midsummer. When gram-negative organisms were recovered by reisolations, pathogenicity was tested by inoculation to disease-free Ricard plants. None of the inoculated plants developed disease symptoms nor were pathogenic isolates recovered. Tests were made with the following species: GRAMINEAE—*Zea mays* L., *Dactylis glomerata* L.; CYPERACEAE—*Carex* sp.; LILIACEAE—*Lilium regale* Wils., *L. canadense* L., *Hostea* sp., *Hemerocallis* sp.; AMARYLLIDACEAE—*Narcissus poeticus* L.; IRIDACEAE—*Iris kaempferi* Sieb., *Gladiolus hybridus* Hort.; SALICACEAE—*Salix babylonica* L., *Populus alba* L.; URTICACEAE—*Urtica gracilis* Ait., *Ulmus americana* L., *Cannabis sativa* L.; POLYGONACEAE—*Rumex acetosella* L., *Polygonum persicaria* L., *Fagopyrum esculentum* Gaertn.; CHENOPODIACEAE—*Chenopodium album* L., *Atriplex patula* L., *Beta vulgaris* L.; AMARANTHACEAE—*Amaranthus* sp., *Celosia* sp. var. Golden Fleece; NYCTAGINACEAE—*Mirabilis nyctaginea* Mac-M.; CARYOPHYLLACEAE—*Dianthus* sp.; PORTULACACEAE—*Portulaca oleracea* L.; RANUNCULACEAE—*Delphinium* sp., *Aconitum napellus* L., *Paeonia officinalis* L., *Nigella* sp., *Thalictrum* sp.; PAPAVERACEAE—*Chelidonium majus* L., *Eschscholtzia californica* Cham., *Papaver orientale* L.; CRUCIFERAE—*Brassica oleracea* L., *Barbarea vulgaris* R. Br.; CRASSULACEAE—*Sedum spectabile* Boreau; SAXIFRAGACEAE—*Ribes* sp., *Heuchera* sp., *Hydrangea* sp.; ROSACEAE—*Malus* sp., *Rosa* sp., *Fragaria* sp.; LEGUMINOSAE—*Robina pseudoacacia* L., *Lupinus polyphyllus* Lindl., *Vicia faba* L.; EUPHORBIACEAE—*Euphorbia pulcherrima* Willd.; BALSAMINACEAE—*Impatiens* sp., *Balsam* sp.; MALVACEAE—*Althaea rosea* Cav., *Abutilon Theophrasti* Medic., *Hibiscus* sp.; LYTHRACEAE—*Lythrum salicaria* L.; ONAGRACEAE—*Oenothera* sp.; UMBELLIFERAE—*Daucus carota* L.; APOCYANACEAE—*Vinca rosea* L., *V. minor* L.; ASCLEPIADACEAE—*Asclepias syriaca* L.; CONVOLVULACEAE—*Convolvulus arvensis* L.; POLEMONIACEAE—*Phlox* sp.; BORRAGINACEAE—*Cynoglossum* sp.; LABIATAE—*Monarda didyma* L., *Salvia* sp., *Leonurus cardiaca* L.; VERBENACEAE—*Verbena* sp.; SOLANACEAE—*Petunia hybrida* Hort., *Nicotiana* sp., *Solanum tuberosum* L., *Lycopersicon* sp.; SCROPHULARIACEAE—*Antirrhinum majus* L.; PLANTAGINACEAE—*Plantago lanceolata* L.; DIPSACACEAE—*Scabiosa* sp.; CUCURBITACEAE—*Cucumis sativus* L.; CAMPANULACEAE—*Campanula* sp.; COMPOSITAE—*Lactuca scariola* L., *Cirsium lanceolatum* L., *Ambrosia artemisifolia* L., *Erigeron* sp., *Arctium lappa* L., *Achillea millefolium* L., *Cosmos* sp. var. Firestick, *Zinnia* sp. var. Peppermint Stick, *Calendula* sp., *Dahlia* sp., *Dimorphotheca* sp., *Tagetes* sp., *Gaillardia* sp., *Centaurea* sp., *Gerbera jamesoni* Bolus., *Chrysanthemum maximum* Ram., *Rudbeckia laciniata* L., *Echinops Ritro* L., and *Heliopsis* sp.

PATHOGEN FACTORS.—*Bacterial isolates.*—Observations of many isolates of *X. pelargonii* indicated that there were differences in ability to produce stem rot or leaf spot. Therefore, single colony isolates 99, 138,

TABLE 2.—*Ability of different isolates of Xanthomonas pelargonii to cause leafspot*

Isolate No.	Symptoms[a] on indicated variety of *Pelargonium hortorum*		
	Radio Red	Ricard	Seedlings
99	0	0	0
138	2	1	0
182	3	2	1

[a] Symptoms indicated as follows: 0, no leaf spots formed; 1, very few leaf spots formed; 2, moderate leaf-spot formation; 3, heavy leaf-spot formation.

and 182 from different geographical sources were compared for relative ability in causing stem rot in cuttings of Radio Red and Ricard and in seedlings of *P. hortorum*. Seventy-five plants, 25 of each host variety, were stem-inoculated with each of the 3 isolates. Results taken 4 weeks after inoculation showed that isolate 182 was most virulent, whereas isolate 99 was the least pathogenic of the 3 (Fig. 1A).

Leaf-inoculation tests were used on the same host varieties after several successive dilution-plate, single-colony transfers of isolates 99, 138, and 182. Isolate 182 again proved to be the most pathogenic, causing leaf spot on all 3 hosts. Isolate 99 did not cause leaf spot on any of the 3 varieties (Table 2). Variability in producing leaf spot was more striking than variability in causing stem rot.

Effect of age of cultures on virulence.—Stock cultures of isolate 182 of *X. pelargonii* in potato-dextrose broth were kept at 9° C for 1, 3, 6, and 12 months. Fresh cultures in potato-dextrose broth were started from the stock cultures of different ages, and stem inoculations were made in Ricard plants. Ten plants were inoculated with each culture and the experiment was repeated with 2 more sets of plants. All inoculated plants developed stem rot regardless of the age of bacterial stock cultures used. When the same cultures were used for leaf inoculation, however, there was a decrease in reaction with increasing age of bacterial stock culture. Plants inoculated with bacterial suspensions from 1- and 3-month-old cultures developed typical bacterial leaf spot, whereas those inoculated with suspensions from 6- and 12-month-old cultures seldom produced symptoms.

ENVIRONMENTAL FACTORS.—*Relation of night temperatures to disease development.*—Frequent observations indicated that pelargoniums at our latitude suffer from stem rot mostly during the summer months. Later, with declining temperatures, disease development is slower than in the summer and the general appearance of the plants is considerably improved. These observations indicate that temperature has a decisive influence on disease development. Experiments were devised to gather data on this point.

TABLE 3.—*Stem rot development in inoculated pelargonium plants (var. Better Times) subjected to different night temperatures*

Weeks after inoculation	Average disease indices[a] at indicated temperatures			
	10°C	16°C	21°C	27°C
1	0	0	0	18
2	0	0	2	37
3	2	3	8	84
4	3	10	12	100
5	8	19	59	100
11	62	58	83	100

[a] Each entry is the average index for 25 plants. Index based on the following classification: 0 = healthy, 10 = slight symptoms; 20 = slight darkening of stem; 40 = blackening of stem, shedding of lower leaves; 60 = stem rot, occasional tip necrosis; 80 = severe stem rot; 100 = dead.

The effect of temperature on growth of noninoculated seedlings was determined first. Twenty-five seedlings of *P. hortorum* in 3-in. pots were kept at 27° C night temperature and another group of 25 plants at 21° night temperature. Day temperatures were not under strict control but were never lower than these values. After 4 weeks of treatment, plants at the higher temperature had made less growth than had the other group and had developed chlorotic leaves, whereas plants kept at 21° were normal in appearance.

In a second test, 30 plants (var. Ricard) in 5-in. pots were kept at 27° night temperature and another equal group at 21° night temperature. After 4 weeks of treatment, both groups were stem-inoculated and kept at 21° night temperature. Four weeks after inoculation, the average disease index for the high temperature group was 42 whereas the index for the 21° group was 19, showing that plants predisposed by high night temperature were more susceptible than were those not predisposed.

Four groups of rooted cuttings (var. Better Times), 25 plants each, were stem-inoculated with isolate 138 and exposed to night temperatures of 10°, 16°, 21°,

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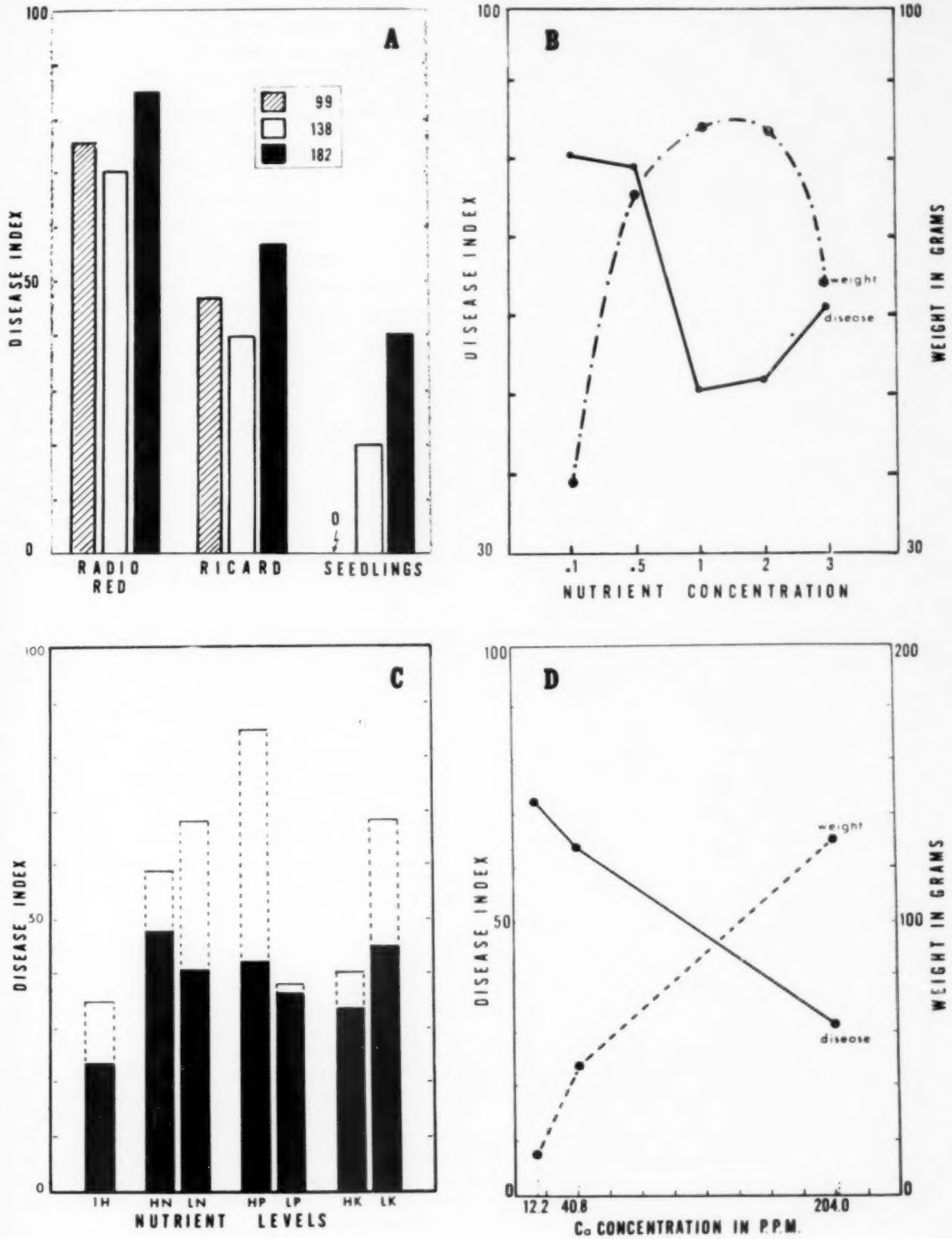
FIG. 1. Stem rot development in pelargonium as affected by bacterial isolates and environmental factors. Disease index is an average value based on the following classification: 0 = healthy; 10 = slight symptoms; 20 = slight darkening of stem; 40 = blackening of stem, shedding of lower leaves; 60 = stem rot, occasional tip necrosis; 80 = severe stem rot; 100 = dead. A) Relative virulence of isolates 99, 138, and 182 of *Xanthomonas pelargonii* to cuttings of the varieties Radio Red and Ricard, and to seedling pelargonium plants. Values are the average readings for 25 plants each taken 50 days after inoculation. B) Comparison of disease development and growth of pelargonium as affected by nutrient concentration. Readings were taken 50 days after inoculation. Fresh weights are those of noninoculated controls. C) Effects of high (H) and low (L) levels of N, P, and K on stem rot development. The basal solution was 1.0 Hoagland's solution (1H). Disease readings were taken 50 days (black) and 90 days (dotted) after inoculation. D) Disease development and growth of pelargonium as affected by Ca in the nutrient solution. Values were taken 50 days after inoculation. Weights are those of noninoculated controls.

and 27° C after inoculation. Four control groups of 25 plants each were stem punctured and the wounds were treated with sterile potato-dextrose broth. Disease readings were taken 1, 2, 3, 4, 5, and 11 weeks after



inoculation (Table 3). Plants kept at 27° showed first symptoms 7 days after inoculation, and all were dead in 4 weeks. In contrast, 3 weeks were required for symptoms to show in plants at 16°, and disease development was still slower at 10°. No control plants became diseased. Although plants at 10° night temperature were slow to show initial symptoms, the disease developed later (Table 3), probably because of an unfavorable temperature for plant growth. Average weights of pelargonium plants after 5 and 11 weeks of growth at the several temperature levels showed that 16° to 21° was optimum for plant growth. In contrast, the causal organism was found to grow best on potato plugs at 24° to 26°.

Relation of nutrient concentration to disease development.—Cuttings (var. Ricard) were rooted in pasteurized sand and later selected for uniformity. Plants were then pretreated by growing in sand culture at the 0.5H nutrient level for 30 days, after which they were again selected for uniformity and arranged in randomized blocks with 5 balanced nutrient levels as treatments (0.1, 0.5, 1, 2, 3 H). After growing in the test nutrient treatments for 30 days, they were stem-inoculated with isolate 182. Noninoculated plants grew best at the 1 and 2 H levels and poorest at the 0.1 H level, as determined by weights at the time of inoculation and 50 days later. Inoculations were made in August.

Disease indices taken 50 days after inoculation showed that disease developed most rapidly at nutrient levels below optimum for growth, somewhat more slowly at levels above optimum, and slowest at 1 and 2 H levels, the levels optimum for growth (Fig. 1,B). Statistically, disease indices did not differ between the 0.1 and 0.5 H treatments and between the 1 and 2 H treatments. The disease index for 1 H was significantly lower than the index for 0.5 H, and 3 H was significantly higher than 2 H (odds 99:1). In other words, disease development increased when plant growth decreased and was least severe with conditions favoring growth (Fig. 1,B).

Relation of nutrient balance to disease development.—High and low levels of nitrogen, phosphorus, and potassium were compared with the 1 H treatment in tests with plants grown for 30 days in their respective treatments before being inoculated in January. Night temperature in the greenhouse during the preinoculation period was kept at 21° C and was held at 24° following inoculation. Average weight of plants at the time of inoculation was 29, 22, 19, 28, 26, 28, and 22 g for plants growing in basal, high N, low N, high P, low P, high K, and low K, respectively. Average weights of noninoculated control plants 110 days after inoculation day were 136, 123, 107, 123, 120, 125, and 115 g, respectively, for the series. Disease indices were recorded 50 and 90 days after inoculation.

Statistical analysis of disease indices taken 50 days after inoculation showed statistically significant differences between balanced and unbalanced treatments, with disease developing most slowly in plants with the balanced nutrients. Of the unbalanced solutions, high N and low K gave highest disease indices, followed by low N and high P (Fig. 1, C). Disease indices of plants given high K and low P were not significantly different, and disease development was slower with these treatments than with any other unbalanced treatments. During the second half of the experiment, disease development was faster in high-P plants than in any of the others (Fig. 1,C).

Rooted cuttings (var. Ricard) were grown in sand at low, medium, and high levels of Ca as described earlier. At the time of inoculation (June), average weights of the plants were 12, 21, and 37 g for low, medium, and high Ca, respectively. The experiment was terminated 50 days after inoculation, when the average weights of noninoculated plants were 16, 46, and 127 g for low, medium, and high Ca, respectively. The weight of Ricard plants increased linearly with increasing calcium in the nutrient solution (Fig. 1,D). Statistical analysis of disease indices showed significantly less (odds 99:1) disease development at high-Ca level than at the 2 lower levels, which were not significantly different. The development of disease increased with a decrease in plant growth in this experiment as in the previous experiments (Fig. 1,D).

Discussion.—All varieties of *P. hortorum* are susceptible to stem rot caused by *X. pelargonii* when tested by stem inoculations; however, varieties differ in resistance, since disease develops much more slowly in some varieties than in others. In commercial culture, the difference in resistance is sometimes more apparent than real. For example, many growers are sure that the variety Radio Red has high resistance and that the varieties Better Times and Ricard are extremely susceptible. In inoculation experiments by Munnecke (7), Radio Red was highly susceptible and Ricard moderately so, whereas Better Times was one of the most resistant varieties tried. Present observations are in accord with those of Munnecke. Differences between observed resistance and experimental results may be attributable to differences in the incidence of latent infections. Better Times, being somewhat resistant, may under certain conditions have a high level of latent infection, and such infections result in many diseased plants when conditions change. Radio Red is very susceptible and under the proper conditions may have few latent infections, since diseased plants may tend to be eliminated.

Formerly, leaf spot and stem rot were assumed to be caused by different species of bacteria (2). More recently, experiments have shown that leaf spot can be caused by the stem rot pathogen (4, 7), although some workers never find leaf spot. The fact that certain isolates of the pathogen will cause leaf spot and stem rot, whereas other isolates cause only stem rot, may help to explain some of the past confusion.

Variation among bacterial isolates in ability to cause leaf spot is even greater than variation in ability to cause stem rot. Several factors may contribute to the uniformly positive results from stem inoculation. Inserted cotton plugs act as reservoirs of inoculum protected from drying. Success in leaf

inoculations, in contrast, depends more on environmental conditions. Such factors may partially explain the decrease in virulence of an isolate (during storage) when tested by leaf inoculations but not when tested by stem inoculations.

Stem rot increased with increasing temperatures from 10° to 27° C during the first 5 weeks after inoculation. Later, the disease increased rapidly in plants kept at 10° night temperature. Since the optimum temperature for growth of *X. pelargonii* is about 27° C and the optimum temperature for host growth somewhat below 20°, disease development during the first few weeks seems to be primarily an effect of temperature on the pathogen. The later increase of disease in the low-temperature plants may be caused by an unfavorably low temperature that weakens the host. Comparison of growth rates of rooted cuttings and disease indices supports this assumption. The effect of high (27°) night temperature on stem rot development is useful for detecting pelargonium plants with latent infections.

Responses to nutrient concentrations also indicated that slow-growing plants develop disease more rapidly than do plants under optimum conditions for growth. Bacterial stem rot of pelargoniums behaves like the typical vascular diseases in its response to nutrients (3, 12), the disease expression being approximately inversely proportional to plant growth. In other respects, however, the pelargonium pathogen is not a typical vascular invader, since apparently no macroscopic symptoms develop until the organism reaches parenchyma tissue. In infected pelargonium stems, the first visual symptom often appears near the stipules and at leaf-scars, which suggests that broken ends of vessels may serve as exits for bacteria. Once in the parenchyma, the causal organism destroys tissue at a relatively constant rate, and the weakest host succumbs first.

Disease in plants grown in unbalanced nutrient solutions was again found to be correlated with decreased plant growth. High-potassium plants behaved similarly to high-calcium plants in stem rot development. It is generally known that actively growing young tissues are rich in potassium and that there is an accumulation of soluble organic nitrogen (amino acids and amides) in potassium-deficient plants (13). Since amino acids and amides are utilized readily by *X. pelargonii* (2), their presence in low-potassium plants may favor growth of the pathogen.

Plants in high-phosphorus solution developed disease slightly faster than did those in low-phosphorus solution, but disease later increased rapidly in high-phosphorus plants. Nightingale (10) found that the absorption of inorganic nitrogen was depressd when available phosphates in the rooting medium were high. Plants grown under such conditions matured early. Current experiments have shown that old pelargonium plants and low stem portions are somewhat more susceptible to *X. pelargonii* than are young plants and top stem portions, respectively. Fast aging and onset of senility may be the reason for fast stem rot development in high-phosphorus plants during the latter part of the experiment.

Plants in high-nitrogen solution developed symptoms more rapidly than did plants in low nitrogen. *X. pelargonii* is known to utilize nitrogen in the organic form (2, 4, 11). Everything else being equal, one could thus expect fastest disease development in plants highest in organic nitrogen. Nightingale (9), working with tomato plants, found that these contained very little organic nitrogen and no nitrate or ammonium when grown in minus-nitrate solutions, whereas plants grown in plus-nitrate solutions had substantial amounts of organic nitrogen. Gallegly and Walker (3), working under similar conditions with tomato plants, found that at 24°C plants in high-nitrogen solutions were more susceptible to bacterial wilt than were plants in low-nitrogen solutions.

The data presented here have immediate practical uses. Infection by *X. pelargonii* may remain latent under conditions favorable for growth of pelargonium. Thus, propagating stock can become contaminated. When plants are exposed to conditions unfavorable for host growth, such as high temperatures or unfavorable nutrient conditions, the latent infections are expressed as stem rot, and infected plants can be eliminated. In practice, it has been found that such stock will remain free of *X. pelargonii* if care is exercised to prevent recontamination.

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ETIOLOGY OF THE DEAD-ARM DISEASE OF GRAPEVINES[1]

T. S. Pine[2]

SUMMARY

The morphological and cultural characteristics of the fungus causing the dead-arm disease of grapevines in California were investigated, and comparisons were made with isolates of the pathogen collected in New York, Canada, and South Africa. These studies showed that the binomial *Fusicoccum viticolum* Reddick should be rejected in favor of *Phomopsis viticola* Sacc.

Increasing the source of carbon in synthetic media altered the cultural characteristics of the different isolates but uniformly increased the production of scolecospores over that of pycnidiospores in all the isolates. The scolecospores formed lateral protrusions after 4 or more days on water agar, on nutrient agar, or in water.

The dead-arm disease of grapevines has become increasingly important during the past decade in the table-grape areas of the northern San Joaquin Valley in California. In years of severe outbreaks, yields sometimes are only 50 per cent of the normal. All the vines in some vineyards of the Lodi district are infected. Although table-grape varieties have been attacked for many years, wine varieties are showing increased incidence of severe disease symptoms, and the disease is spreading southward into important raisin-growing areas. Because of this increased activity of the pathogen, it became important to study the causal organism, disease development, and control measures in an effort to determine their relationship to the pathogen responsible for the disease elsewhere in the world. The present paper deals with research on the causal organism.

The disease was first reported by Reddick in 1909 as occurring in many of the grape-growing areas of New York (20). By 1922 it was found in Michigan (1) and Kansas (29) and 2 years later in South Carolina and Ohio (2). Hiura described the disease as "root-neck blight" in Japan in 1924 (16), whereas Drayton (7) mentioned in 1926 that it had been growing in importance since 1921 in Ontario, Canada. In 1935, Miles (18) reported dead-arm in Mississippi. Hewitt (15) recorded it from California, and du Plessis (8) found the disease in South Africa. The following year van Poeteren (27) noted a similar disturbance on glasshouse grapes in Holland, and in 1937 Goidànich (9) described dead-arm in the Trieste region of Italy.

THE CAUSAL ORGANISM.—Growth of the septate, hyaline, branching mycelium of the fungus causing the dead-arm disease of grapevines in California is concentrated in the cortex of shoots, petioles, tendrils, and cluster stems, generally 3–4 cell layers beneath the epidermis. It can move through all the leaf tissues, and, in green berries, it forms in the hypodermal parenchyma. Within these areas, pycnidia may arise; these are set off indefinitely from the host tissue by a thick pseudoparenchymous stroma. The single pycnidial cavity is at first discoid, becoming more or less globose as the body erupts through the epidermis (Fig. 1). The ostiole may be regular or lacking, the opening then being an irregular tear. The entire inner surface of the pycnidium is lined with sporophores of 2 types: 1) straight, apically pointed, 12–20 $\times$ 2 μ, bearing hyaline pycnidiospores (α spores) that are single, elliptical-fusoid, 8–10 $\times$ 3 μ, acute at 1 or both ends, and generally 2 guttulate; 2) short and straight, 5–8 $\times$ 1.5 μ, bearing at their tips hyaline scolecospores (β spores), that are filiform, hemate to flexuous, 22–30 $\times$ 1–1.5 μ (Fig. 1). The lining of the pycnidium may be convoluted, but it is not sufficiently so to form a locular wall. Mature pycnidia range from 200 to 350 μ in diameter. Both types of spores are exuded from the pycnidium in a thick, water-soluble matrix that forms chracteristic yellow-white spore horns. The host epidermis surrounding the erumpent black pycnidia becomes darkened, then bluish, and eventually bleaches (Fig. 2).

The perfect form of the fungus has not been observed in nature or in culture in California.

On potato-dextrose agar (PDA), growth was at first white but became cream or tan at maturity. Sectoring occurred commonly. Pycnidia were sometimes scattered singly over the surface but more generally coalesced to form large, black, multilocular bodies. Cultures of the fungus derived from single spores produced definite mycelial, conidial, and intermediate types of colony growth, as reported for other members of the Sphaeropsidales by Hansen (14). Colonies of these types growing on PDA are illustrated in Figure 2.

The fungus was observed and named *Fusicoccum viticolum* by Reddick in 1909 (20). This genus was chosen instead of *Dothiorella* since Reddick, at the time, believed the scolecospores to be paraphyses, and *Dothiorella* was described as lacking paraphyses. In 1911, Shear (26) found a Pyrenomycete associated with the dead-arm fungus, which he tentatively called *Cryptosporella viticola* (Reddick) Shear. Single-ascospore cultures of this form gave pycnidia "practically identical" with those produced from pycnidiospores of *F. viticolum* taken from diseased grape vines; however,

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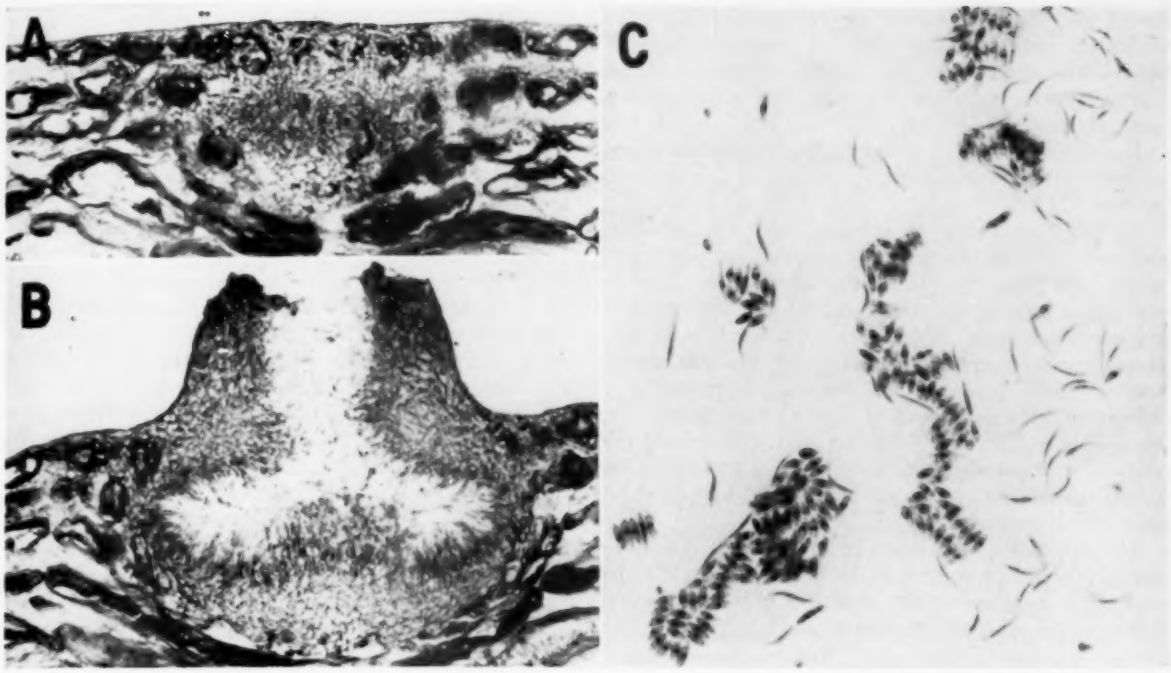

FIG. 1. A, B) Pycnidium of the dead-arm fungus on grapes, illustrating 2 stages of development. A) Early growth in the subepidermal tissue. B) Mature pycnidium, showing detached spores and the convoluted base of the chamber (180×). C) Spores (α and β) of the dead-arm fungus (240×).

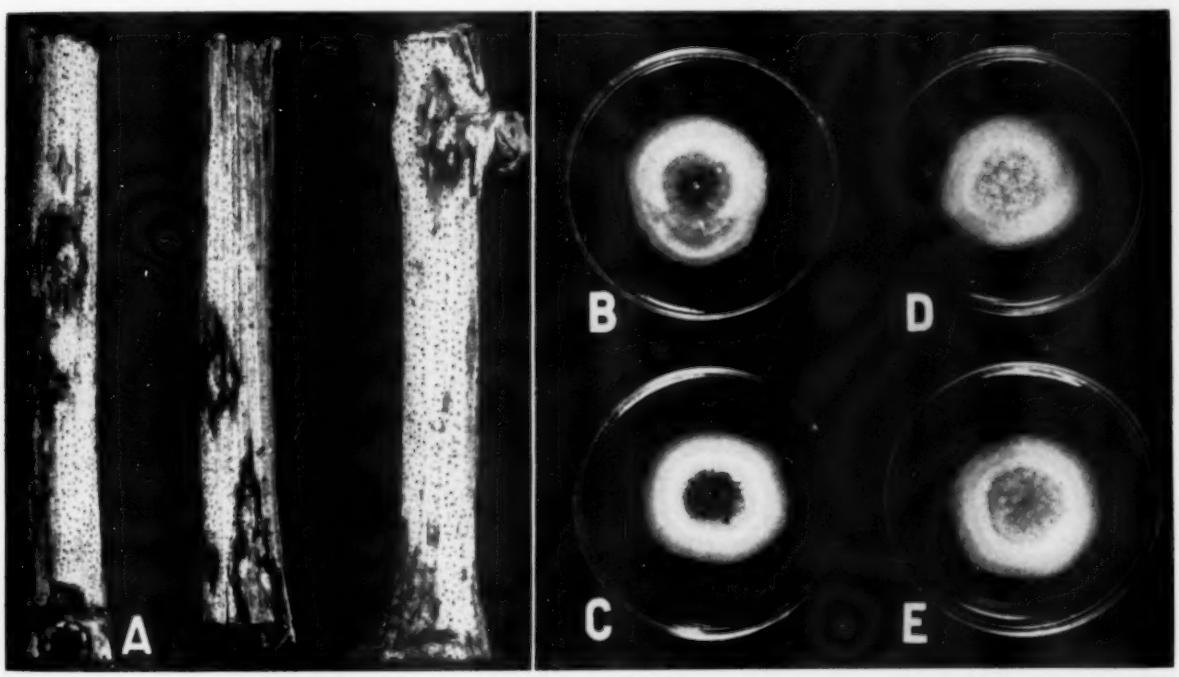

FIG. 2. A) Segments of grape canes covered with pycnidia of the dead-arm pathogen. The host epidermis became stained bluish-black, then bleached; this is characteristic of infection (2×). B—E) Single-spore cultures of the California isolate, grown on potato-dextrose agar. B) Mycelial type of growth. C) Conidial type. D, E) Intermediate growth types.

Shear was not able to grow the perfect stage from the imperfect, and he attempted no inoculation experiments with ascospores. No subsequent report of the Cryptosporella stage, either in nature or in culture, has been noted.

Later, Reddick (21) accepted Shear's nomenclature, as well as the latter's interpretation of the scolecospores.

In 1937, Goidànich (9) re-examined the morphological and cultural characteristics of the pathogen and altered the name to *Phomopsis viticola*. This change was based upon the presence of 2 pycnidiospore types, typical of the genus *Phomopsis*. It seems evident that this generic change, from *Fusicoccum* to *Phomopsis*, was entirely correct. The combined research of Diedicke (6), Das Gupta (5), Grove (12), Hahn (13), von Höhnel (17), and Wehmeyer (28) has defined the genus *Phomopsis* in such a way that little doubt remains concerning the forms that should be placed in this very large genus.

The binomial *Phomopsis viticola*, however, has been pre-empted by Saccardo. In 1880, Saccardo (22) described a fungus which he called *Phoma viticola*. Later, after erecting the genus *Phomopsis* (23), he changed the name to *Phomopsis viticola* (24). Grove, in 1917 (10), observed the same fungus in England and gave it the same name. This repetition was realized by Grove in 1919 (11), and the authority for the binomial was thereafter given as Saccardo.

Saccardo's description of *P. viticola*, restated in 1931 (25), essentially fits the observations of the dead-arm fungus by Reddick (20, 21), Coleman (4), du Plessis (8), Grove (10, 12), Goidànich (9), and the present writer. It should be noted that Saccardo's 1915 description was based upon material collected by H. D. House in New York and Massachusetts, this being the same area in which Reddick was working at the time. Also, Grove's 1919 description of *P. viticola* var. *ampelopsidis*, which differs from *P. viticola* only in its lack of ability to color the host epidermis, was taken from material collected in New Jersey by J. B. Ellis.

Since there are no discrepancies between the original description of *P. viticola* by Saccardo and the observations made of the dead-arm fungus, as it occurs in California, the name *Fusicoccum viticolum* Reddick is rejected in favor of *Phomopsis viticola* Sacc.

CULTURAL STUDIES.—To extend studies of this patho-

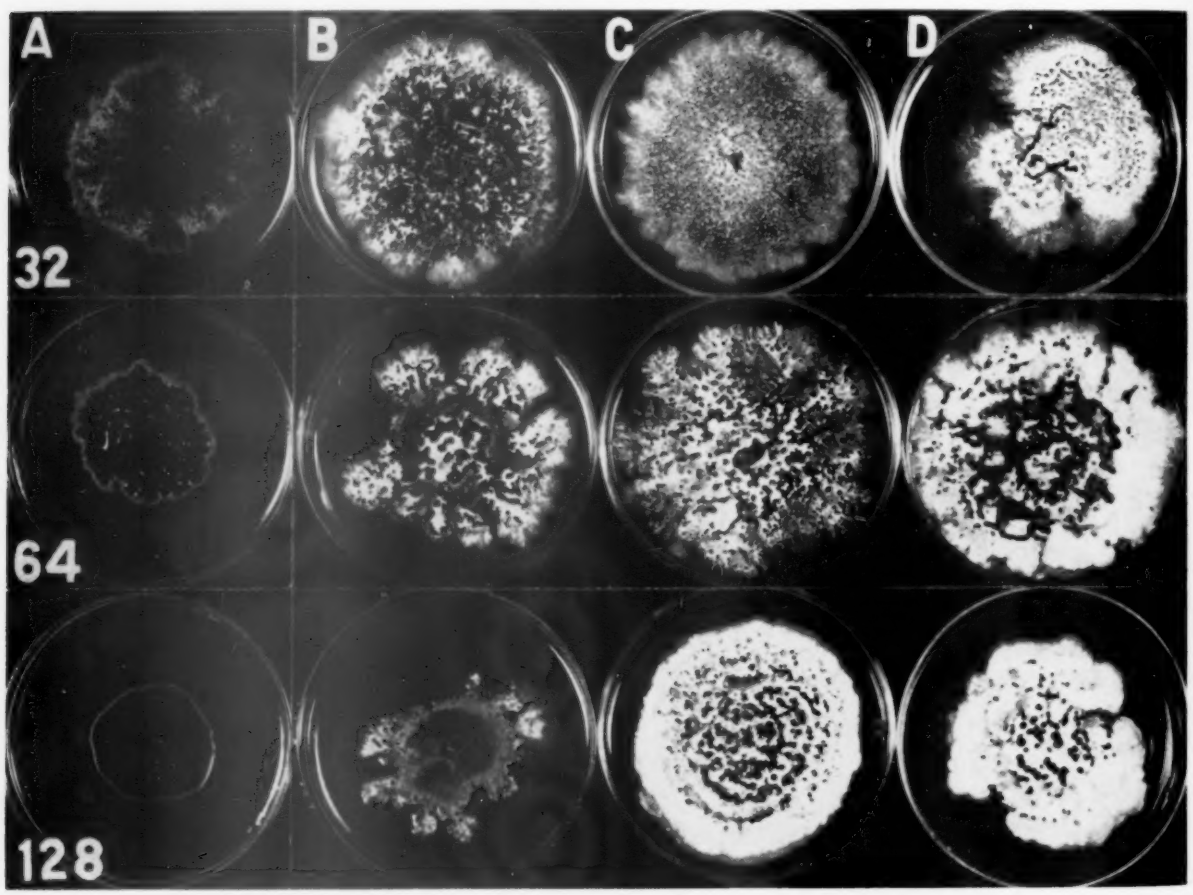

FIG. 3. Four isolates of *Phomopsis viticola* grown on media containing 32, 64, and 128 g dextrose per liter. A) New York isolate. B) California isolate. C) Canadian isolate. D) South African isolate.

FIG. 4. β spores of *Phomopsis viticola*, with lateral protrusions produced after 7 days on water agar. The protrusions varied from barely visible to about 6 μ. Spores were derived from pycnidia growing on sterilized grape canes (450×)

gen to isolates from beyond the grape-growing areas of California, isolates of the fungus from Canada (Ontario), South Africa, Italy, and New York were collected for comparison.[3] The Italian isolate, from the original collection of Goidànich, was found to be sterile and could not be used in these studies. Recently, Dr. D. F. Millikan, Jr., submitted an isolate from Missouri for comparison; this was found to be the same as the Canadian isolate.

When the Canadian, New York, South African, and Californian isolates were grown on PDA, on prune, cornmeal, nutrient, oatmeal, and malt agars, and on autoclaved grape canes, the rates of growth, color, and shape of mycelial mats, time and abundance of pycnidial formation, and maturation of spores were found to be the same for all isolates. When pycnidia of the different isolates, from growth on sterile grape canes, were embedded in paraffin and sectioned, no differences could be found in their morphological characteristics. Averages of spore sizes (both α and β types) were the same as for the California isolate. Germinating α spores from the different isolates were indistinguishable.

These studies, and the fact that observations on the symptomatology and pathogenesis of the dead-arm disease and its causal agent by workers in Canada, South Africa, and New York are in agreement, are evidences that 1 pathogen is involved, *Phomopsis viticola* Sacc.

The production of β spores by the different isolates on PDA and on sterile grape canes varied from less than 1 per cent to as much as 25 per cent. In order to gain them in larger quantities, the technique of Nitimargi (19) was repeated. The basic nutrient used contained 0.75 g magnesium sulfate, 1.25 g potassium phosphate, 2 g asparagin, and 15 g agar in 1000 ml of distilled water. To this was added dextrose at 16, 32, 64, and 128 g per liter. Single-α-spore transfers of the 4 isolates were made to Petri dishes or to tubes of this medium. At the end of 6 weeks, water mounts were made of mature pycnidia, and the relative amounts of α- and β-spore production studied.

No differences were found in the cultures on media containing 16 or 32 g per liter of dextrose, β-spore production averaging 10 per cent or less for all isolates. At 64 g per liter of dextrose, α and β spores were produced in almost equal numbers; at 128 g, at least 90 per cent were β spores in all cultures. At the 64- and 128-g levels, some of the α spores showed a considerable enlargement of the guttrels, to the extent that some spores were almost globose and had lost their original identity. At 128 g of dextrose, α spores occasionally were exuded from the pycnidium with their sporophores attached. The β spores retained their original appearance, except for a tendency to become slightly thicker than usual in their central portion.

With the increase in carbon there was an appreciable change in the cultural characteristics of the 4 isolates. The New York strain became greenish-black,

[3] For their help in securing the introduced cultures, the author is indebted to Dr. R. S. Dickey, Cornell University, Ithaca, New York; Dr. G. C. Chamberlain, Department of Agriculture, Ottawa, Canada; Professor Antonio Ciccarone, Instituto di Pathologia Vegetale, Catania, Italy; and, Dr. Chris Orffer, University of Stellenbosch, Union of South Africa.

grew less than at lower levels of carbon, but produced large quantities of a thick, glistening oily substance. The California strain grew very slowly, became dark brown in color, and formed fewer but larger pycnidia than at lower levels of carbon. The Canadian isolate produced many small pycnidia at 32 g dextrose, but these became fewer and larger at 128 g. The South African isolate changed the least of all, yet its pycnidia were larger and fewer at the higher concentrations than at the lower (Fig. 3).

During examination of water mounts of β spores from the cultures on media containing 128 g of dextrose per liter, it was noted that lateral protrusions were being produced by these spores after about 4 days. These protrusions varied in size from mere bumps barely visible to 2–3 μ (Fig. 4). Many of the β spores became decidedly granular in appearance, the interior of the cell showing from 1 to 9 discrete dark areas within a textured protoplasm. The protrusions always arose at a point in the cell wall opposite one of these dark areas. After 7 days, some protrusions had reached 6 μ, approximately 1 quarter the length of the spore, and were about the same width as the β spore. In any 1 mount, about 30 per cent of the β spores showed some sort of protrusion. Cross walls were not observed, either in the protrusions or in the β spores, nor was any branching noted. A few of the spores produced 2 protrusions, either on the same or opposite sides. When 2 β spores rested side by side, the protrusions sometimes grew toward the adjacent spore and at other times away from it.

Since this phenomenon had first been observed with β spores taken from high-nutrient cultures, it was thought that the effect might be residual in nature. Therefore, spore collections were made from pycnidia grown on sterilized grape canes or PDA, and spore dilutions in distilled water made to water agar plates. In this way, the α and β spores were well separated on the plates, and the rapid germination and growth of the α spores did not obscure the much slower activity of the β spores. Protrusions were again produced by the β spores to the same extent and after the same length of time as previously observed. The same action was found to occur when the spores were placed on plates of nutrient agar.

The role of β spores in the life cycle of *Phomopsis* spp. has been studied by many workers (3, 4, 6, 10, 12, 13, 17, 19, 26). The production of β-spore protrusions is undoubtedly the same phenomenon observed by Cayley (3), who worked with the Phomopsis stage of *Diaporthe perniciosa* Marchal; however, Cayley lost her original culture and was not able to duplicate the experiment. Whether or not this action is to be considered germination of the β spore remains in question. No attempts have been made to explore the environmental conditions necessary to produce more extensive "growth."

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THE BEAN ROOT ROT COMPLEX IN IDAHO[1]

Parul Chatterjee[2]

SUMMARY

Five different genera of fungi were isolated from bean plants affected with root rot. All of the isolates that were demonstrated to be prime pathogens were identified as *Fusarium solani* f. *phaseoli*. There appeared to be 3 distinct physiologic and pathogenic types within this species. A histological study of infected plants revealed that *F. solani* f. *phaseoli* is capable of entering plants by direct penetration, through the stomata on the hypocotyl, and through wounds. The mycelium developed within and between the cells of the cortex and endodermis but did not penetrate the pericycle except in the case of very young branch roots.

Dry root rot is considered to be the major unsolved bean disease problem in the state of Idaho. It is so prevalent that it is almost impossible to find a noninfected plant growing in the major bean-producing areas. Although losses are minimized by good cultural practices, it is believed that yields could be substantially increased if effective control measures were available. Extensive tests have been conducted in recent years (6) to determine feasibility of controlling the disease by soil applications of fungicide-fumigants, but results have been so variable that practical recommendations cannot be made. It has been postulated that the varied responses obtained from soil treatment might be due to changing complexes of pathogenic organisms. This study was initiated to determine for Idaho soils the number and identity of pathogens that cause root rot symptoms and the pathological relationship of each to the bean plant.

MATERIALS AND METHODS.—In order to obtain representative samples of pathogenic organisms from naturally infested soil, a large batch of soil was removed from a bean field near Twin Falls during the late summer of 1955 and stored in the greenhouse at Moscow. This soil was kept at nearly constant temperature and moisture levels throughout the duration of the experiments. Bean plants were grown in this soil in the greenhouse during the winter of 1955, and isolations were made from those with disease symptoms. During the summer of 1956, isolations were made from field-grown plants from the Twin Falls area. In the course of this study, 324 isolates were obtained and identified. For purposes of identification, a subculture derived from a single spore was obtained from each isolate, and all were grown under identical conditions of medium, light, temperature, humidity, and time. The keys of Barnett (1) and Wollenweber and Reinking (10) were used for making initial identifications. Three of the pathogenic subcultures of Fusarium were later submitted to W. C. Snyder for positive identification.

A pure culture of each isolate was tested for pathogenicity to Red Mexican beans in the greenhouse. To accomplish this, seeds of Red Mexican beans (UI-34) were planted in pasteurized soil at the rate of 4 per 6-in. pot. Noninoculated controls were used in every experiment, and special care was taken to prevent contamination by watering. Inocula of the isolates were prepared either by culturing on sterilized liquid media (9) or on plates of Difco potato-dextrose agar. In the former method, the mycelial mats from the liquid media were removed by aseptic filtration and dispersed into 500 ml of sterile distilled water. The soil was infested by adding 50 ml of this suspension to each pot. When agar plates were used for culturing the isolates, inocula were prepared by flooding each plate with 15 ml of sterile distilled water. The spore suspension thus obtained from each plate was then added to a pot of soil. Each pot was infested at the time the seed was planted. Disease readings were made on sample plants removed from the pots at intervals of 3, 6, and 8 weeks after planting.

A modification of the method described by Kilpatrick et al (5) was used to study the development of disease symptoms. In the present study, surface-disinfested bean seeds (UI-34) were germinated in paper towels and the seedlings transferred to pint Mason jars. The seedlings were supported in the jars on artificially infested filter papers, and a small quantity of sterile Hoagland's solution was added to support growth during the test period. Each isolate was tested separately in order to compare the development of symptoms. To determine the mode of entrance of the pathogens, their distribution within the host tissues, and the host response, histological sections of diseased tissues were made either freehand or after the tissues were embedded in paraffin. When the paraffin method was used, the tissues were fixed in formalin-acetic-alcohol and dehydrated with dioxane or tertiary butyl alcohol. They were embedded in Fisher's Tissuemat (m. p. 56°–58°C) and stained with a combination of safranin and fast green (4) or of safranin and picroaniline blue (3).

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EXPERIMENTAL RESULTS.—Approximately 77 per cent of the isolates obtained from diseased bean plants were *Fusarium* spp. (Table 1); most of these were of the Elegans and Martiiella types as defined by Wollenweber and Reinking (10), but not all of them were pathogenic. Species of *Rhizoctonia* were isolated occasionally from 3- to 6-week-old plants and frequently from more mature plants. Other fungi such as species of *Alternaria*, *Gliocladium*, and *Pythium* were less frequently obtained, but none of these proved to be pathogenic in these tests.

Table 2 records in summary the data obtained from the pathogenicity tests. These tests were made with cultures developing from single spores of each isolate. All isolates, of each species, that were judged to be identical on the basis of cultural characteristics and pathogenicity tests were assigned a common code number for easy reference.

It will be noted that 4 groups of Fusarium isolates, 115, 116, 129, and 133, produced disease but that the degree of virulence was not the same for each. The disease symptoms were very severe in plants inoculated with isolates of group 116; 2 of the plants inoculated died 3 weeks after inoculation. All others had severe necrosis on belowground parts, and the root systems were mostly decayed by the end of 6 weeks. The plants remained alive, however, by virtue of adventitious roots that formed on the stem near the soil surface.

Isolates of group 115 were slightly less virulent than those of group 116. A considerable amount of yellowing of the leaves was observed on a majority of the plants inoculated with these isolates. Decay developed on the underground portions of plants inoculated with group 115 but not as rapidly as on those of plants inoculated with group 116. Fusarium isolates in group 133 were least pathogenic, and those of group 129 were intermediate. Isolates of group 122 (*Rhizoctonia solani*) were mildly pathogenic but had little effect on growth.

It was observed also that plant growth was more severely impaired by some isolates than by others. The reduction in growth of plants inoculated with isolates of groups 115, 116, and 129 was approximately proportional to the severity of disease symptoms, but the growth of plants inoculated with isolates of group 133 was greater than that of the noninoculated controls.

A representative subculture of each of the 3 groups of *Fusarium solani* f. *phaseoli*, 115, 116, and 129, was selected for further pathogenicity studies. These tests were designed to determine the effect of age of the host plant at the time of inoculation on the severity of symptoms. In some of these trials, healthy 4-week-old plants were transplanted to infested soil. In the case of older plants, inoculation was accomplished by introducing the inoculum into the soil in which the plants were growing; this was done when the plants began to set pods. In all cases, typical disease symptoms developed 7–10 days following inoculation. These results demonstrated that neither susceptibility to infection nor host response was materially altered by age of the host.

TABLE 1.—*Frequency of isolation and identity of 324 isolates from root rot diseased beans grown in naturally infested soil*

Identity of fungi isolated	Frequency of isolation
Pythium sp.	5
Fusarium sp. (Sec. Elengans)	27
F. solani f. *phaseoli* (Burk.) Snyd. & Hans.	170
F. oxysporum Schlecht	52
Rhizoctonia sp.	37
R. solani Kühn	13
Gliocladium sp.	17
Alternaria sp.	3

The symptoms of the bean root rot disease have been described by various authors (2, 7, 9). In this study, an attempt was made to study the development of symptoms in plants grown in infested hydroponic solutions in glass jars. The first observable symptoms on the bean plants developed 6–10 days after the seedlings were transplanted to the jars. The typical reddish discoloration in the form of lesions and streaks developed on hypocotyls and tap roots. The lesions and streaks gradually increased in size and turned reddish brown to brown. In no case was a definite margin of the lesions observed. These initial symptoms developed on any region of the root epidermis. They were observed in the ruptured zone where the lateral roots emerged but were not confined to this

TABLE 2.—*Pathogenicity on Red Mexican UI No. 34 bean plants grown in greenhouse of 324 isolates of fungi from naturally infested soil*

Code No.	Identified organism	No. of isolates tested	No. plants tested	Disease severity rating average[a]	Aver. length of plant (in.)[b]
106	*Pythium* sp.	5	20	0	
104	*Fusarium* sp. (Sec. Elegans)	27	108	0	
108	*F. oxysporum*	11	44	0	
115	*F. solani* f. *phaseoli*[c]	51	204	4	14.0
116	*F. solani* f. *phaseoli*[c]	47	188	5	9.3
117	*Rhizoctonia* sp.	37	148	0	
122	*R. solani*	13	52	1	24.7
124	*Gliocladium* sp.	17	68	0	
129	*F. solani* f. *phaseoli*[c]	72	288	3	17.0
133	*F. oxysporum*	41	164	1	52.1
138	*Alternaria* sp.	3	12	0	
	Control			0	26.3

[a] 0—None; 1—very mild; 3—moderate; 4—severe; 5—very severe (7).

[b] This measurement was made from the ground line to the tip of the growing point. Each figure is an average of 6 replicates of 4 plants each.

[c] *Fusarium solani* f. *phaseoli* were grouped here in 3 different types because their degree of pathogenicity and physiological behavior on culture media were different. W. C. Snyder (personal correspondence) identified these isolates as *F. solani* f. *phaseoli* and considered the diversity as normal for the species.

area. Histological data supported this observation by showing direct penetration of the pathogens through the epidermal cells. In plants inoculated with isolates of groups 115, 116, and 129, the brown lesions and streaks spread longitudinally and horizontally and finally encompassed the root and part of the hypocotyl. This was observed as soon as 3 weeks after transplanting. In plants inoculated with isolates of groups 115 and 116, the lateral roots often became shriveled and nonfunctional as the disease progressed. Isolates of groups 122 and 133 did produce lesions and streaks, but other than a slight longitudinal enlargement no further change was observed.

All isolates of Fusarim penetrated the host plant in a similar way. Mycelium was observed on the surface of the underground parts of the hypocotyl and roots. Penetrations occurred directly through the epidermis of the lateral roots as well as through the opening formed by the emergence of lateral roots. In direct penetration, the hyphae penetrated the thin cuticle without the formation of a visible appressorium-like structure. After penetration, the hyphae became a little swollen under the epidermis but soon assumed a normal diameter. There was a tendency for the hyphae to grow in parallel strands after penetration. These strands were observed both intracellularly and intercellularly. In some cases, however, the hyphae branched and formed knot-like masses in the epidermal cells.

The epidermis, cortex, and endodermis of the tap roots and the underground part of the hypocotyl ruptured because of the emergence of lateral roots. The mycelium, present in the soil and on the surface of the underground parts of the plants, invaded the cortical cells at these points. From these points of invasion, the pathogens often progressed into the newly developed lateral roots.

Occasionally, the pathogen was observed to penetrate through the stomata on parts of the hypocotyl at the soil level. No penetration of hyphae was observed through either root hairs or hairs on the hypocotyl. In 3 instances, isolates of group 116 produced typical symptoms at the cotyledonary attachment. The hyphae were found in the cortical cells only for a few millimeters above and below the cotyledonary node. This observation was not sufficient to indicate whether or not the penetration took place through the cotyledonary attachment.

Following all these penetrations, nuclei of epidermal and cortical host cells did not seem to have attracted the hyphae. In the early stages of disease development, the nucleus was observed frequently in close association with hyphae without showing any disturbance. The penetration of the hyphae and their early development in the parenchyma cells of the host did not cause any disorganization of the cytoplasm that could be detected by differential staining.

The activity of the pathogen was greatest in the cortical cells of the tap roots and underground parts of the hypocotyl. Here the hyphae branched and progressed longitudinally as well as laterally. In 10–12 days after penetration, the cortex was filled with mycelium. Cross sections of infected tissue at this stage showed considerable amounts of hyphae in the cells and in the intercellular spaces. A brown substance developed in the cells and more frequently in the intercellular spaces of the heavily infected cortex and was responsible for the characteristic pigmentation of the infected tissues. Microchemical tests for lignin, suberin, and wound gum (4, 8) failed to indicate the nature of this brown substance. In severe cases of infection, the hyphae in the cortical cells of the host extended as far as 1 in. above the soil line, although the symptoms were not always apparent more than ¼ in. above the soil. The pathogens also invaded the endodermal cells, where the hyphae were intracellular. The pericycle, conducting tissues, and pith of primary roots were free of invasion.

Isolates of groups 115 and 116 produced 3 kinds of spores within and on the host tissues. These spores were observed when the underground parts of the plants started to decay. Chlamydospores were observed in the collapsed cortical cells, and microconidia and macroconidia were produced on the outer surface of the cortex.

The direct penetration of lateral roots was similar to that of the tap root and hypocotyl. It has been noted that the fungus hyphae often invaded lateral root tissues from the cortex of the tap root. The pericycle, which is the origin of the secondary roots, was never observed to be infected; however, the meristematic tissues of the root initials, including procambium, often were infected. This is probably the origin of infection in the vascular tissues of the lateral roots. A longitudinal section of a young lateral root showed the presence of long hyphae in the phloem tubes and xylem vessels. It was mentioned previously that some of the lateral roots became shriveled and nonfunctional as the disease progressed. This morbidity probably was due to the presence of hyphae in the vascular elements.

The penetration of host plants by isolates of group 122 (*Rhizoctonia solani*) occurred in the same way as previously described for the Fusaria. Part of the cortex near the point of penetration was invaded by hyphae. The activity of the hyphae in this case was very restricted, and they never penetrated as far as the endodermis. As a result of this restricted activity, lesions were always small.

Discussion.—Two hundred and forty-nine of the 324 fungus isolates obtained from root rot lesions of beans were species of the genus *Fusarium* and 50 were species of *Rhizoctonia*. Two hundred and twenty-four of the Fusarium and 13 of the Rhizoctonia isolates were pathogenic to some degree under the conditions of these tests, but only 98 of the Fusarium and none of the Rhizoctonia isolates were virulent pathogens. It is possible that all of the isolates might have caused disease symptoms if the plants were predisposed to other conditions.

The isolates that were determined to be *F. solani* f. *phaseoli* (Burk.) Snyd. & Hans. were segregated into

3 distinct types based upon their differences in virulence and growth characteristics. Although variability of this type is a characteristic of many species of *Fusarium*, the constancy of behavior that subcultures of these isolates displayed indicates that these variations have a genetic basis and suggests the occurrence of physiologic races within this species. A better understanding of the relationships among these organisms seems necessary before we can work out satisfactory control procedures.

The histological pathology of bean root rot was first investigated by Burkholder (2), but he did not report on the mode of penetration. In the present study, hyphal penetration was observed to take place directly through the epidermal cells, through the stomata of the hypocotyl, and through wounds. The host and parasite lived in apparent harmony immediately after the initiation of infection, but a considerable amount of plasmolysis was observed as the mycelial development became extensive in the cells. The mycelium grew quite readily both within the cells and in the intercellular spaces. Following the initiation of infection, a brown-colored substance was detected in the cells and intercellular spaces of the cortex. The chemical nature of this substance could not be determined by microchemical tests. It was not definitely established whether this brown substance was produced by the pathogen or whether it was a reaction product of the host in response to attack; however, in plants inoculated with mild strains of the pathogens, the brown deposits in the cells and intercellular spaces seemed to prevent the spread of mycelium. It seems probable, therefore, that this is a host response and that virulent strains of the fungus developed so rapidly that the host did not respond rapidly enough to prevent envelopment. It is noteworthy that the fungus did not penetrate stelar tissues inside the pericycle, except in the case of young developing lateral roots where penetration of all tissues was very rapid.

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ROOT AND STEM ROT OF SOYBEAN CAUSED BY PHYTOPHTHORA SOJAE N. SP.[1]

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SUMMARY

An undescribed species of *Phytophthora* was found associated with root and stem rot of soybeans in Illinois. Comparative studies indicated that Illinois isolates were identical with isolates obtained by other investigators from similarly diseased soybeans in Ohio and North Carolina. This pathogen causes pre-emergence and postemergence damping-off and a root and stem rot of plants at all stages of maturity. In Illinois, stem rot is the most conspicuous symptom. A comparison of 8 inoculation techniques revealed that 2 gave quick reliable results. These were 1) inoculation by introduction of infested whole oats into the soil at the time of planting and 2) insertion of bits of mycelium into seedling hypocotyls. Both methods gave nearly 100 per cent infection in susceptible varieties whereas little or no disease developed in resistant varieties. Comparable checks remained healthy. The pathogen also caused a damping-off of alfalfa and sweetclover seedlings.

This fungus appears distinct from previously described species of *Phytophthora* and the name *Phytophthora sojae* n. sp. is proposed. It differed in morphology, pathogenicity, growth rate, and staining reaction from the 2 closely related species, *P. cactorum* and *P. megasperma*.

Phytophthora root and stem rot of soybean, *Glycine max* (L.) Merr., was first reported from Ohio and North Carolina in 1955 (6, 7, 8). It now has been recognized in Illinois, Missouri, and Indiana, as well as in Ontario, Canada.[3] The causal organism has been considered to be *Phytophthora cactorum* (Leb. & Cohn) Schroet. (4, 6). The disease may be serious on both heavy and light sandy soils and is favored by cool, rainy weather. It is most severe in areas of fields in which water may stand but also appears in scattered spots on well-drained areas. It attacks soybean plants in all stages of growth, causing pre-emergence and postemergence damping-off and a root and stem rot that results in wilting and death of plants from the primary-leaf stage to maturity. Under certain conditions it may also cause a seed rot. Stems of plants attacked in the primary-leaf stage first appear water-soaked, then the leaves wilt, and the entire plant dies and turns brown. The symptom usually noticed first on older plants is a wilting of the leaves. Chlorotic areas appear between the veins and along the margins of the leaves, and a brown canker advances up the stem, frequently as far as the node of the second or third trifoliate leaf. At times lesions may be confined entirely to the stem, with the lower portion of the stem and the roots having no perceptible symptoms. The pod and stem blight fungus *Diaporthe phaseolorum* var. *sojae* (Lehm.) Wehm. has been found fruiting on stems shortly after they had been killed by the soybean Phytophthora. In Illinois, stem rot is more conspicuous than is root rot.

Herr (4) found young plants to be more susceptible than older plants. On older plants, the symptoms were slower in appearing than on young plants, and a longer time was required for the plants to succumb to the disease. Schmitthenner and Williams (5) described a method of artificial inoculation in which 7-day-old plants growing in a quartz sand culture in 16-oz. paper cups were inoculated by injecting 2- to 3-day-old shake cultures of the fungus through the side of the cup with a hypodermic needle. Bernard et al (1) found that resistance to this disease is inherited by a single dominant gene.

This paper describes studies on the morphology, taxonomy, and host range of the causal organism, and a comparison of inoculation techniques is given.

MATERIALS AND METHODS.—For isolation of Phytophthora from soybeans, 2- to 3-in. lengths of stems containing the advancing margin of fungus decay were surface sterilized in 25 per cent Clorox (5.25 per cent sodium hypochlorite) for 1½ to 2 minutes and then rinsed in distilled water. Short radial sections of tissue were then cut from the margins of the cankers, placed on Petri plates of potato-dextrose agar or of cornmeal agar, and incubated at 20°C. Five isolates were obtained from widely scattered areas in Illinois. A North Carolina isolate was obtained from Dr. C. B. Skotland, Raleigh, North Carolina, and an Ohio isolate was obtained from Dr. A. F. Schmitthenner, Ohio Agricultural Experiment Station, Wooster, Ohio.

Other species of Phytophthora used were *P. megasperma* Dreschs., *P. cactorum* (Leb. & Cohn) Schroet. (a lemon and a lilac isolate), *P. erythroseptica* Pethyb., and *P. infestans* (Mont.) d By., all obtained from Dr. F. A. Weiss, Curator, American Type Culture Collection; *P. cactorum* (a sweetclover isolate), obtained from Dr. D. W. Benedict, Harrow, Ontario; and *P. cryptogea* Pethyb. & Laff., obtained from Dr. D. C. Erwin, Department of Plant Pathology, University of California.

Culture media used were potato-dextrose agar (PDA), Difco cornmeal agar (CMA), hemp seed in

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[3] Personal correspondence.

TABLE 1.—*Emergence and damping-off of Harosoy soybeans planted in soil infested with the soybean Phytophthora by means of various methods*

Inoculation method	In infested soil			Checks[b]	
	No. seeds planted	No. emerged	No. damped-off[a]	No. planted	No. emerged
1. Mycelial suspension poured over the seed	48	18	5	48	25
2. Infested corn-meal-sand medium mixed with soil	48	0	0	48	42
3. Infested whole-oat medium mixed with soil	48	0	0	48	37
4. Seed soaked 6 hours in a mycelial suspension	48	21	4	48	29

[a] Postemergence damping-off.

[b] Checks subjected to the same treatment as were the corresponding treated seed except that the Phytophthora inoculum was omitted.

water, cornmeal-sand, whole oats, and toothpicks impregnated with CMA. Hemp seed in water was prepared by adding 3 or 4 seeds to a culture tube containing 10–15 ml distilled water and autoclaving the tube for 20 minutes at 15-lb. pressure. Cornmeal-sand medium consisted of equal parts by volume of yellow cornmeal and freshly washed quartz sand mixed together and steamed for 1 hour. Whole oats for whole-oat medium were boiled 20 minutes in water. Both the cornmeal-sand and whole-oat media were placed in 250-ml Erlenmeyer flasks, 200 ml per flask, and autoclaved 1 hour at 15-lb. pressure on each of 2 successive days. Small pieces of toothpicks on which the fungus was to be cultured were boiled 1 hour in distilled water and then placed in CMA and autoclaved 30 minutes at 15-lb. pressure. They were then removed aseptically and placed on the surface of solidified CMA in a Petri plate.

Mycelial suspensions used for inoculation consisted of two 2-week-old Petri plate cultures of the fungus on CMA mixed with 100 ml distilled water in a Waring Blendor.

Seed was surface sterilized by immersion for 1½ minutes in 25 per cent Clorox. Soil to be sterilized was placed in greenhouse flats or pots and steamed for 3 hours on each of 2 successive days.

Morphological measurements were taken from cultures of the fungi on hemp seed in water. All measurements were made with the fungi mounted in water.

EXPERIMENTS AND RESULTS.—*Inoculation methods.*—The methods of inoculation compared may be divided into 2 groups: those in which soil or seed was infested by various techniques at the time of planting (Methods 1–4), and those in which the treatment was applied to 10-day-old seedlings (Methods 5–8). Twelve seeds of the variety Harosoy were planted per treatment for each inoculation method and check. The experiment was replicated 4 times.

In Method 1, soil was infested by pouring 100 ml of a mycelial suspension of the fungus over the seed in the soil in each pot. Check pots were watered with an equal amount of sterile CMA suspension. Inoculum for Method 2 consisted of the fungus grown on cornmeal-sand medium. Two hundred ml of the inoculum was mixed with the soil of the upper half of each pot. The same amount of sterile cornmeal-sand medium was mixed with the soil of each check pot. Inoculum for Method 3 consisted of the fungus grown on whole oats. The inoculum was mixed with the soil in the upper half of each pot at the rate of 200 ml per pot. An equal amount of sterile whole oats was mixed with the soil of each check pot. In Method 4, the seed was soaked in a mycelial suspension for 6 hours previous to planting. Seed of checks was soaked in a sterilized CMA suspension the same length of time. In Method 5, the fungus was grown on small splinters of toothpicks impregnated with CMA. An infested piece of toothpick was inserted into the hypocotyl of each seedling and covered with petroleum jelly. A sterile piece of toothpick was inserted into the hypocotyl of each check plant. In Method 6, roots of seedlings that had been grown in sterilized sand in a greenhouse flat were dipped into a mycelial suspension of the fungus, and the seedlings were then transplanted into soil. Roots of check plants were dipped into a sterilized CMA suspension before transplanting. In Method 7, trenches were dug adjacent to the seedlings by slicing a sterilized spatula through the soil at the bases of the stems. One hundred ml of a mycelial suspension of the fungus was poured into the trenches in each pot, and the trenches were then filled in with soil. Trenches of check pots were filled with the same amount of sterile CMA suspension and then closed. In Method 8, pieces of mycelium from a culture of the fungus on hemp seed in water were inserted into a longitudinal slit in the hypocotyl of each seedling. Checks were wounded with a sterile needle. All wounds were covered with petroleum jelly to prevent drying out.

Results were recorded 2 weeks after inoculation (Tables 1, 2). Two of the methods involving infestation at the time of planting, namely, soil infestation

TABLE 2.—*Incidence of disease in Harosoy soybean seedlings inoculated in various ways with the soybean Phytophthora*

Inoculation method	No. of plants inoculated[a]	Percentage killed
5. Infested toothpicks inserted into hypocotyl	44	95
6. Roots dipped into a mycelial suspension	48	2
7. Mycelial suspension poured into a trench adjacent to the plants	46	2
8. Wound inoculation with mycelium	42	93

[a] From 40 to 48 seedlings were used as checks for each treatment. None of these became diseased. The check seedlings were treated like the inoculated seedlings except that the Phytophthora inoculum was omitted.

with the fungus grown on 1) cornmeal-sand medium or on 2) whole oats, were highly effective. No plants emerged in pots treated by either of these methods, whereas good emergence occurred in the check pots. When a mycelial suspension of the fungus was poured over the seed or when seed was immersed in a mycelial suspension before planting, many plants escaped in treated pots. Moreover, emergence was less in the comparable check pots than in other check pots. When 10-day-old seedlings were inoculated by inserting infested toothpicks or bits of mycelium into the hypocotyls (Fig. 1, A), nearly all seedlings were killed whereas all check plants remained healthy. Nearly all plants inoculated by dipping the roots into a mycelial suspension or by pouring a mycelial suspension into a trench at the bases of stems still appeared healthy at

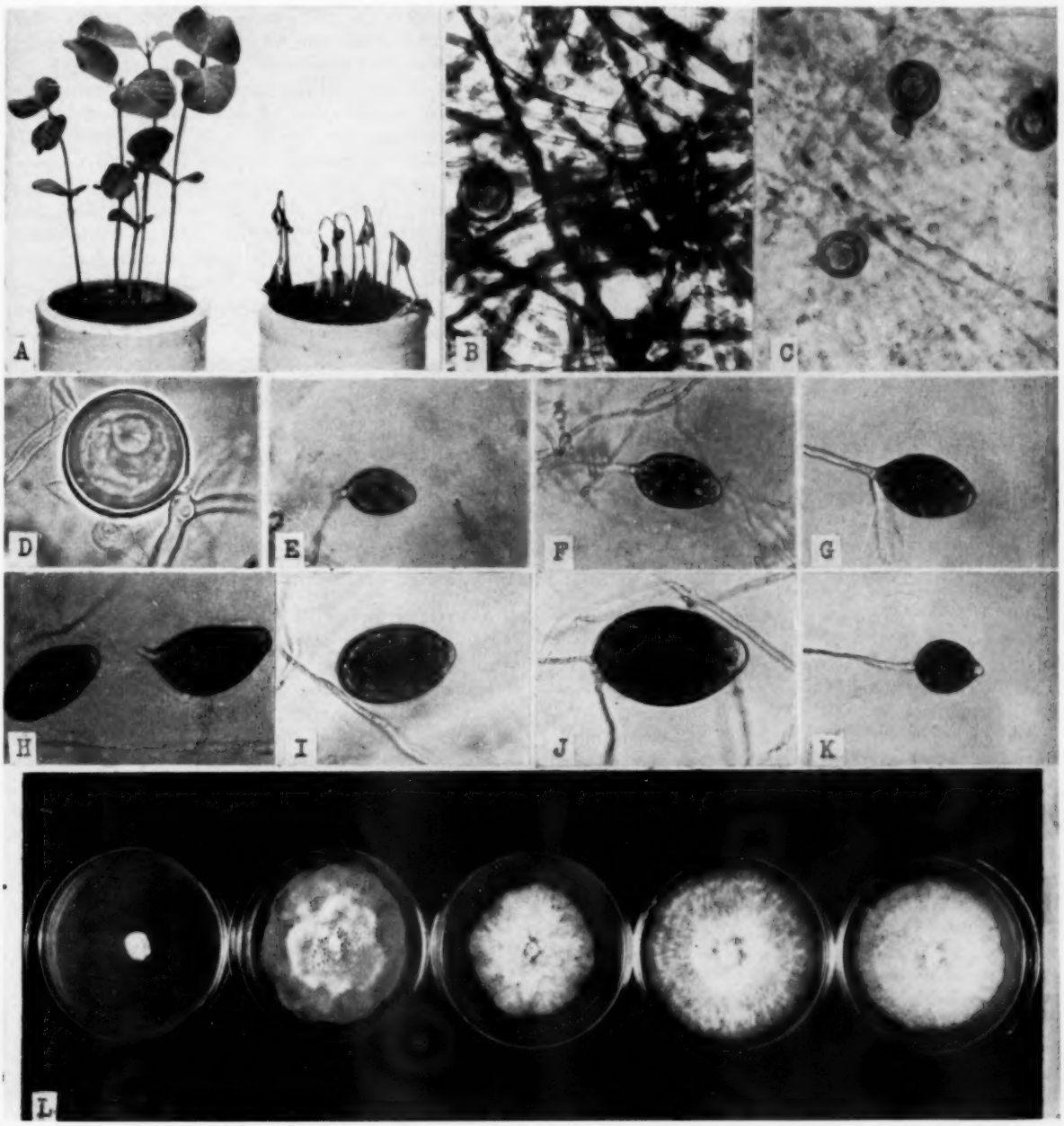

FIG. 1. A) Fifteen-day-old Harosoy soybean plants 5 days after inoculation by the wound method: noninoculated left, inoculated right. B) Mycelium of *Phytophthora sojae* treated with chloriodide of zinc (×270). C) Mycelium of *P. cactorum* treated with chloriodide of zinc (×270). D) Oogonium and paragynous antheridium of *P. sojae* (×450). E-J) Sporangia of *P. sojae* (×270). K) Sporangium of *P. cactorum* (×300). L) Left to right: Seven-day-old cultures of *P. sojae*, *P. megasperma*, and sweetclover, lemon, and lilac isolates of *P. cactorum* on potato-dextrose agar.

the conclusion of the experiment.

Inoculations by planting in soil infested with the fungus grown on a cornmeal-sand medium or on whole oats and inoculation by inserting mycelium into hypocotyls were each tested in supplementary experiments in which both resistant and susceptible varieties were used to determine whether or not these methods were too severe for screening plants for resistance. In 1 experiment, seeds of the susceptible variety Harosoy and the resistant variety Illini were planted at the rate of 12 seeds per pot in soil infested with the fungus grown on cornmeal-sand medium and in soil infested with the fungus grown on whole oats. Twelve seeds of each variety were also planted in check pots for each of the 2 methods. The experiment was replicated 5 times. Results were recorded 2 weeks after inoculation (Table 3).

In pots infested with the fungus grown on whole oats, all plants of the susceptible variety Harosoy were killed. Plants in check pots (sterile whole oats added) remained healthy. The resistant variety Illini grew nearly as well in the infested pots as in the check pots. In pots infested with the fungus grown on cornmeal-sand, the mortality rate was high among resistant seedlings. Therefore, infestation of soil with whole-oat inoculum appears to give the more reliable test for resistance.

Inoculation by insertion of mycelium (from hempseed cultures) into hypocotyls was tested in an experiment in which 2 susceptible varieties, Harosoy and Hawkeye, and 2 resistant varieties, Monroe and Illini, were used. Fifty-two 10-day-old seedlings of each of the 4 varieties were inoculated. Results were recorded 1 week after inoculation. All plants of each of the 2 susceptible varieties became infected, and all died within 7 days. All plants of the resistant variety Monroe remained healthy; 51 Illini plants remained healthy whereas 1 plant was killed. This method gives a very clear-cut distinction between resistant and susceptible plants.

Artificial inoculation by insertion of fungus mycelium into hypocotyls or by planting in soil infested with the fungus grown on whole oats may be recommended as simple and effective. Inoculation by inserting mycelium into the hypocotyls has the advantage of eliminating confusion resulting from poor germination of the seed. Plants may be inoculated immediately upon emergence, and resistant plants are not affected; however, if large plant populations are to be screened, the use of soil infested with the fungus grown on whole oats is the better method. With either method, reliable results may be obtained in 7 to 14 days.

Nature of disease resistance.—In a study of the nature of disease resistance, stems of the resistant variety Illini and of the susceptible variety Harosoy cut into sections 1½ to 2 in. in length were sterilized either by autoclaving in distilled water or by propylene oxide gas. A transfer of the soybean Phytophthora was introduced into tubes containing the stems in water. The fungus grew equally well on stems of both the resistant and the susceptible variety. Stems and leaves of each of these 2 varieties were also frozen and then ground in a meat chopper. The resulting pulp was squeezed through cheesecloth, and the juices obtained were centrifuged at 8,000-10,000 G to remove much of the suspended material. These liquids were filtered through diatomaceous earth in a Büchner funnel and then sterilized by filtration through bacterial filters. The sterile liquids from the 2 varieties were each mixed with equal volumes of 2 per cent water agar cooled to 45–50°C and poured into Petri plates. The fungus grew as well on the extract from the resistant variety as on that from the susceptible variety.

The possibility that the fungus produced toxins that contributed to the cause of death of susceptible soybean plants also was investigated. Ten-day-old seedlings of both the resistant variety Illini and the susceptible variety Harosoy were placed in liquid from hemp seed cultures of the soybean Phytophthora that had been sterilized by filtration through Seitz filters. Two types of checks were included: stems of each variety placed in liquid from hemp seed in water in which no fungus had been cultured and stems of each variety placed in distilled water. Three replications—3 bottles with 3 stems each—were included for each variety in the treatment solution and in each check solution. No wilting of seedlings occurred in either treatment or in the check solutions.

Host range.—The pathogenicity of the soybean Phytophthora was tested on red clover, sweetclover, Ladino clover, alfalfa, lespedeza, birdsfoot trefoil, corn, oats, wheat, green bean, garden pea, Lima bean, and mung bean. Soybeans were included as a check on the viability of the inoculum. The soil was steam sterilized and infested at the time of planting by pouring 50 ml of a mycelial suspension onto the seed in each 18-in. row. Fifty surface-sterilized seeds of each species were planted in each of two 18-in. rows in both infested and non-infested soil. Seed in check flats was watered with the same concentration of sterile CMA in water. Thirty-five soybean seedlings, 20 alfalfa seedlings, and 5 sweetclover seedlings damped-off; there was no damping-off of the other species (Table 4). A stem rot occurred in a single green bean plant, but all other species remained healthy. Emergence of some

TABLE 3.—*Incidence of disease in a resistant and in a susceptible variety of soybeans planted in soil infested with the soybean Phytophthora by 2 different methods*

Material added to soil	No. of surviving plants from 60 seed planted of indicated variety	
	Harosoy (susceptible)	Illini (resistant)
Infested whole oat medium	0	40
Noninfested whole oat medium	52	44
Infested cornmeal-sand medium	0	16
Noninfested cornmeal-sand medium	58	47

TABLE 4.—*Percentage emergence in 14 crops sown in soil infested with the soybean Phytophthora*

Crop	Variety	Percentage emergence	
		Inoculated	Check
Red clover (*Trifolium pratense* L.)	Kenland	67	70
Ladino clover (*T. repens* L.)		59	67
Sweetclover (*Melilotus alba* Desr.)	Spanish	58	59
Alfalfa (*Medicago sativa* L.)	Ranger	81	78
Lespedeza (*Lespedeza stipulacea* Maxim.)	Climax	41	51
Birdsfoot trefoil (*Lotus corniculatus* L.)	Viking	89	89
Wheat (*Triticum aestivum* L.)	Prairie (C. I. 12371)	61	84
Oats (*Avena sativa* L.)	Clinton	98	99
Corn (*Zea mays* L.)	Ind. WF9× C. I. 187-2	100	100
Mung bean (*Phaseolus aureus* Roxb.)		51	98
Lima bean (*P. limensis* Macf.)	Baby Fordhook	14	43
Green bean (*P. vulgaris* L.)	Burpee's Stringless Green-Pod	82	94
Garden pea (*Pisum sativum* L.)	Little Marvel	89	85
Soybean (*Glycine max* (L.) Merr.)	Harosoy	69	80

crops was significantly less in the infested flats than in the checks, however, and rotted seed and partially germinated dead seed of green bean and Lima bean were observed. Ten-day-old seedlings of corn, mung bean, Lima bean, and garden pea inoculated by insertion of the fungus directly into the bases of the stems remained healthy, but inoculated soybeans were killed.

This fungus does not appear to be a serious threat to any crops commonly grown in rotation with soybeans. Suhovecky (7) reported that Clinton oats, Ranger alfalfa, Kenland red clover, Ohio C54 corn, Thorne wheat, and timothy are not susceptible to the fungus. With the exception of alfalfa, the results obtained in these studies are in agreement with Suhovecky's findings.

The pathogen.—When cultured on PDA, the soybean Phytophthora grew mainly on the agar surface, with the mycelium forming a dense, thick, white colony in which folds appeared after a few days (Fig. 1, L). The margin of the colony was definite and usually undulating. Fruiting bodies were not produced on this medium. On CMA, most growth occurred below the surface of the agar, and there were relatively few aerial hyphae. Oogonia, antheridia and oospores appeared in abundance after 1–2 weeks, but sporangia were not observed in cultures grown on this medium. Hyphal swellings were common. Growth on hemp seed in water was entirely submerged; it was white and cottony and filled the diameter of the culture tube, growing upward as much as 1½ to 2 cm after 2 weeks. Oogonia and antheridia and oospores formed in great numbers after 1–2 weeks. Sporangia appeared very sparsely, but when the culture was poured into a Petri plate numerous sporangia appeared in 1 to 2 weeks. Hyphal swellings were common.

Mycelial growth of the fungus was typical of that in the genus *Phytophthora*. Hyphae exhibited characteristic right-angle branching with slight constrictions at the bases of branches. Hyphae were 3–9 μ in diameter, mostly 4–6 μ. Hyphae in young cultures were coenocytic, but many hyphae in old cultures had no protoplasm and numerous septa were present. Hyphal

TABLE 5.—*Comparative morphological characteristics*[a] *of Phytophthora cactorum, P. megasperma, and the soybean Phytophthora*

Organism	Oogonium		Oospore		Oospore wall		Sporangium		
	Range	Average	Range	Average	Range	Average	Range	Average	Apex[b]
	μ	μ	μ	μ	μ	μ	μ	μ	
P. cactorum									
Lilac isolate	19.9-33.3	28.9	17.5-29.2	25.4	0.8-1.6	1.2	14.9-46.6 × 11.6-36.6	34.2×27.4	D
Sweetclover isolate	24.9-37.5	30.4	22.5-29.2	26.6	0.8-1.6	1.4	24.2-41.6 × 20.8-32.5	32.4×26.5	D
P. megasperma		48.8		42		3.8	35-60 × 25-43		I
Soybean Phytophthora	28.3-45.8	36.9	19.2-38.3	31.4	1.3-3.3	2.4	23.3-88.8 × 16.6-51.8	58×38.3	I

[a] Based on measurements of 75 oogonia, oospores, and oospore walls and 125 sporangia. Sporangia of *P. megasperma* were not observed; the figures given are from Dreschler's original description.
[b] D—distinct papillae, I—broad, indistinct papillae.

swellings were common in old cultures. They were spherical or ellipsoidal, terminal or intercalary, and usually single, but sometimes crowded. Chlamydospores were not produced on any media.

Oogonia were typically globose and did not have a funnel-shaped base (Fig. 1, D). The spherical oospores had thick, smooth walls and nearly filled the oogonia. A total of 194 oogonia and oospores were measured (Table 5). The oogonia varied from 28.3 to 45.8 μ in diameter, with an average of 36.9 μ. Oospores varied from 19.2 to 38.3 μ in diameter, with an average of 31.4 μ. Two hundred and sixty-six oospore walls were measured; they varied in thickness from 1.3 to 3.3 μ, with an average thickness of 2.4 μ. Antheridia were paragynous and were usually attached to the oogonium near the oogonial stalk.

Sporangia were borne terminally on simple or sparsely branched sporangiophores. They were typically ovoid or ellipsoid in shape and had a broad and usually inconspicuous birefringent apical thickening or papilla of the type often called nonpapillate. Blackwell (2) is doubtful that any species of *Phytophthora* produces a completely nonpapillate sporangium and suggests that it would be more correct to designate the condition usually referred to as nonpapillate as inconspicuously papillate. Sporangiophores resumed growth by branching outside and below the sporangium. A total of 168 sporangia were measured; sizes were extremely variable, ranging from 23.3 to 88.8 μ in length and from 16.6 to 51.8 μ in width. The average length and width were 58 and 38.3 μ, respectively. The range in size and shape of sporangia and papillae of the soybean Phytophthora is illustrated in Figure 1, E–J.

Sporangia germinated either directly, by the formation of a germ tube, or indirectly, by the production of zoospores. When germination occurred by means of a germ tube, the tube usually grew from the tip or side of the apex. The zoospores were biflagellate and formed within the sporangium. As the fully developed zoospores were moved, a very thin and delicate membranous vesicle formed; it expanded and quickly ruptured as the zoospores moved into it. The zoospores immediately swam around, but soon settled down and formed spherical cysts.

The Ohio, the North Carolina, and the 5 Illinois isolates of the soybean Phytophthora were compared to determine whether or not they are distinguishable. The temperature-growth relations and growth rates of the isolates were compared on CMA incubated at 5°, 10°, 15°, 20°, 25°, 30°, and 35°C. Maximum growth of all isolates occurred at 20°. None of the isolates grew at either 5° or 35°. After 7 days, plates that had been incubated at 5° and 35° were placed at laboratory temperatures. All isolates incubated at 5° grew, but those incubated at 35° failed to grow. Growth rates of all isolates were about the same except for the North Carolina isolate, which grew slightly faster than the others. Measurements of oospores, oospore walls, and sporangia were essentially the same in all isolates. Sporangia of all isolates had broad, indistinct papillae.

Taxonomy.—The temperature-growth relations of the soybean Phytophthora were compared with the closely related species, *P. cactorum* (a lilac, a lemon, and a sweetclover isolate) and *P. megasperma*. Isolates were grown on CMA, and the experiment was replicated 3 times and repeated once. The isolates of *P. cactorum* and of *P. megasperma* grew at all temperatures except 35°C. Optimum growth of isolates of *P. cactorum* was at 25° in both experiments and for *P. megasperma* at 20°in the first experiment but at 25° in the second.

Growth rates of *P. cactorum* (lilac, lemon, and sweetclover isolates) *P. megasperma*, and the soybean Phytophthora were compared on PDA and CMA at 20° and 25°C, as well as on hemp seed in water at room temperatures. The experiment was replicated 4 times. The isolates of *P. megasperma* and of *P. cactorum* grew much faster than did the soybean Phytophthora on all media. This difference was especially striking on PDA (Fig. 1, L). After 6 days on this medium, colony diameters of *P. megasperma* and *P. cactorum* averaged from 4 to 5 times as great as did those of the soybean Phytophthora. On CMA, radial growth of colonies of *P. megasperma* and of *P. cactorum* was approximately twice as great at 20° and 2–4 times as great at 25° as that of the soybean Phytophthora. Height of growth of colonies of *P. megasperma* and of *P. cactorum* on hemp seed in water in culture tubes was approximately twice that of colonies of the soybean Phytophthora.

The pathogenicity of *P. cactorum* (lilac, lemon, and sweetclover isolates), of *P. megasperma*, and of the soybean Phytophthora were compared on the soybean variety Harosoy. Plants were inoculated by inserting mycelium into hypocotyls of plants 10 and 27 days old. Plants in check pots were wounded without the insertion of mycelium. All wounds were covered with petroleum jelly. There were 5 soybean plants in each treatment, and the experiment was replicated 4 times. All plants of both ages inoculated with the soybean Phytophthora were killed, although the symptoms on the older plants were somewhat slower in appearing and death was less rapid than was the case with younger plants. All plants inoculated with the other species of *Phytophthora* remained healthy.

Oogonia, oospores, oospore walls, and sporangia from hemp-seed cultures of *P. cactorum* (lilac and sweetclover isolates) and of *P. megasperma* were measured and compared with those of the soybean Phytophthora. A total of 75 measurements of oogonia, oospores, and oospore walls and 125 measurements of sporangia were recorded for each isolate (Table 5). The diameter of the oogonium and oospore and thickness of the oospore wall of the soybean Phytophthora were intermediate to those of *P. cactorum* and of *P. megasperma*. Sporangia of the soybean Phytophthora were oval or ellipsoidal, and were usually much larger than sporangia of the 2 isolates of *P. cactorum*, which typically were spherical (Fig. 1, K). In addition, sporangia of the soybean Phytophthora had broad, in-

distinct papillae, whereas sporangia of the isolates of *P. cactorum* had prominent papillae. Sporangia of *P. megasperma* were not observed during this investigation, but Drechsler's (3) description of *P. megasperma* indicates that they are somewhat smaller than those of the soybean Phytophthora and have the same type of broad, inconspicuously papillate apex.

Mycelia of the isolates of *P. cactorum*, *P. megasperma*, *P. cryptogea*, *P. erythroseptica*, and *P. infestans* and of the 7 isolates of the soybean Phytophthora were treated with chloriodide of zinc, a chemical used to test for the presence of cellulose in fungi. Mycelia of all isolates of the soybean Phytophthora, as well as of *P. erythroseptica* and *P. infestans*, gave a strong positive reaction (Fig. 1, B), as indicated by a deep bluish-red stain. Mycelia of *P. megasperma*, *P. cryptogea*, and the 3 isolates of *P. cactorum* stained faintly (Fig. 1, C). Oogonia of isolates of *P. cactorum*, *P. megasperma*, and the soybean Phytophthora also stained faintly, but sporangia of the isolates of *P. cactorum*, as well as those of the soybean Phytophthora, showed a strong reaction.

Discussion.—Tucker (9) recognized 2 species of *Phytophthora* with paragynous antheridia—*P. cactorum* (Leb. & Cohn) Schoet. and *P. syringae* (Kleb.) Kleb. A number of new species with paragynous antheridia have been described since the publication of Tucker's monograph in 1931. In 1956, Waterhouse (10) published a compilation of the original descriptions of all the species that had been placed in this genus.

Among species of *Phytophthora* with paragynous antheridia, distinctions are based upon the diameters of oogonia and oospores; presence or absence of reticulations, undulations, spines, etc. on the oogonial walls; size and shape of antheridia; size and shape of sporangia; and prominence of papillae. Although average size of sporangia is of some significance, ranges may be extreme. Temperature-growth relations are also used to distinguish species, the chief characteristic distinguishing *P. syringae* and *P. primulae* from *P. cactorum* being the failure of *P. syringae* and *P. primulae* to grow when incubated at 25°–28°C.

Hyphal walls of the Phycomycetes that have biflagellate zoospores typically show a positive reaction for the presence of cellulose when treated with chloriodide of zinc. To the writers' knowledge, no comparison of the intensity of staining with this chemical has been made among species of *Phytophthora*. Since the mycelium of some species gives a strong reaction whereas that of others shows only a faint reaction, this test may be of some value in distinguishing species. The difference between stained mycelium of the soybean fungus and that of the related species, *P. cactorum* and *P. megasperma*, is striking.

The soybean fungus may be readily distinguished from *P. cactorum* both by the sexual and by the asexual reproductive structures. Oogonia and oospores of the soybean Phytophthora are larger than those of *P. cactorum*, and its large oval or ellipsoidal sporangia with broad indistinct papillae contrast sharply with the smaller, spherical, prominently papillate sporangia of *P. cactorum*. A review of the literature and illustrations describing species relegated to synonomy with *P. cactorum* by Tucker revealed that all these species were more prominently papillate than the soybean Phytophthora. The sporangia of the soybean fungus are similar to those of *P. megasperma;* however, the soybean Phytophthora has significantly smaller oogonia and oospores than that species. Neither *P. cactorum* nor *P. megasperma* were pathogenic to soybeans. On the bases of morphology, physiology, and pathogenicity, the soybean Phytophthora appears distinct from both *P. cactorum* and *P. megasperma*.

A review of descriptions and illustrations as compiled by Waterhouse (10) of all other recorded species of *Phytophthora* having paragynous antheridia revealed that none of them were sufficiently similar to the soybean isolate to be regarded as the same organism. In comparison to the soybean pathogen, *P. cactorum* var. *applanata* has smaller oogonia and more prominently papillate sporangia. *P. stellata* has smaller oogonia and spines on its oogonial walls, and *P. macrospora* and *P. quininae* have much larger oogonia and oospores. Oogonia and oospores of *P. fagopyri* and *P. cyperi-rotundati* are nearly the same size as those of the soybean fungus, but sporangia of both have more prominent papillae, and antheridia of *P. cyperi-rotundati* may be either amphigynous or paragynous. There are several other species with oogonia and oospores approximately the same size as those of the soybean fungus and whose sporangial apices do not appear to differ greatly from those of the soybean Phytophthora. *P. cyperi-iriae*, with undulate oogonial walls, *P. cyperi-bulbosa*, with reticulate oogonial walls, *P. lepironeae*, with angular and undulate oogonial walls, and *P. verrucosa*, with verrucose oogonial walls, appear to be clearly distinct from the soybean fungus. Differences in morphological characteristics are less obvious between *P. inflata*, *P. porri*, *P. primulae*, *P. syringae*, and the soybean fungus; however, neither the large contorted lobed antheridia of *P. inflata* nor the irregularly thickened oogonial walls of *P. porri* were observed in cultures of the soybean fungus. In addition, antheridia of *P. porri* are amphigynous as commonly as they are paragynous and fasten to nearly any part of the oogonium. *P. primulae* and *P. syringae* are considered distinct, since they fail to grow at 25°–28°C.

The soybean Phytophthora appears to be distinct from all previously described species. All characteristics observed were constant, and several isolates were indistinguishable. Therefore it is described as a new species—*Phytophthora sojae* n. sp.

Phytophthora sojae sp. nov.

Hyphis primo continuis, maturitate demum septatis, ramis augulis rectis patentibus; hyphis a sporangiophoris non distinctis; sporangiis ovoideis vel ellipsoideis 23.3–88.8 × 16.6–51.8 μ, medio 58 × 38 μ, papillis latis non conspicuis, in hyphis vel zoosporis germinantibus; oogoniis sphaericis stipitibus cylindraceis, 28.3–45.8 μ latis, medio 37 μ; oosporis sphaericis, levibus, paene oogonia implentibus, 19.2–38.3 μ latis, medio

31 μ; episporio 1.3–3.3 μ crasso, medio 2.4 μ; antheridiis paragyneis; omnibus partibus in liquore "chloriodide-zinc" fortiter se colorantibus.

Hyphae continuous when young, becoming evacuated and septate with age, giving a strong positive reaction for the presence of cellulose when treated with chloriodide of zinc, ranging in diameter from 2 to 9 μ, mostly 4–6 μ. Hyphae exhibiting typical right-angle branching with slight constrictions at the bases of branches; sporangia borne on simple, undifferentiated, sparingly branched sporangiophores, ovoid or ellipsoid in shape, ranging in size from 23.3–88.8 $\times$ 16.6–51.8 μ, averaging 58 $\times$ 38.3 μ, with a broad, indistinct, birefringent apical thickening or papilla, and germinating directly by germ tubes or indirectly by zoospores; oogonia smooth walled and spherical, ranging in size from 28.3 to 45.8 μ, averaging 37 μ; oospores smooth walled and spherical, nearly filling the oogonia, ranging in size from 19.2 to 38.3 μ averaging 31 μ, oospore walls ranging from 1.3 to 3.3 μ in thickness, averaging 2.4 μ; antheridia paragynous.

Habitat: Roots and stems of soybean plants (*Glycine max*) in Illinois, Ohio, North Carolina, Missouri, and Indiana, U.S.A., and in Ontario, Canada.

The type is represented by a culture isolated from a diseased soybean stem from a field near Blackstone, Illinois, in 1955. Material of the type has been placed in the Herbarium of the University of Illinois, Urbana.

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INHIBITION OF ERWINIA AMYLOVORA BY BACTERIUM SP. ISOLATED FROM FIRE BLIGHT CANKERS[1]

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SUMMARY

A nonpathogenic yellow bacterium, tentatively identified as a species of Bacterium, frequently was isolated together with *Erwinia amylovora* (Burr.) Winsl. et al from fire blight cankers on apple and pear trees. Thirty-five of 40 isolates of *Bacterium* sp. inhibited growth of *E. amylovora* on yeast-extract agar. Sterile culture filtrates from 20 of the 35 inhibitory isolates prevented growth of *E. amylovora* when 1–4 ml of filtrate was added to 6 ml of yeast-extract broth seeded with the pathogen.

Comparisons of inhibitory activity in buffered and unbuffered media showed that *Bacterium* sp. inhibited *E. amylovora* by increasing the acidity of culture media to a degree unfavorable for growth of the pathogen. One isolate appeared to produce an antibiotic inhibiting *E. amylovora*.

The relationship of secondary organisms to the survival of plant pathogenic bacteria in their hosts has had little study. In the case of fire blight of apple and pear, a disease for which effective control measures are lacking, knowledge of antagonistic effects of associated microorganisms on the pathogen might be of practical as well as of theoretical importance.

In 1945, a study of bacteria associated with fire blight cankers was begun at the Ohio Agricultural Experiment Station (unpublished results of R. C. Thomas, H. C. Young, and H. F. Winter). In this work, certain isolates of a yellow bacterium were found to inhibit growth of *Erwinia amylovora* (Burr.) Winsl. et al on agar media, and filtrates from these cultures prevented growth of the pathogen when added to liquid media. Some isolates inhibited fire blight symptoms when introduced into apple twigs with the pathogen. Rosen (6) isolated from fire blight cankers a bacterium that was inhibitory to *E. amylovora* in culture and in pear shoots. The results reported herein deal with the mechanism whereby bacteria associated with *E. amylovora* inhibit the pathogen in culture. An abstract has been published (3).

MATERIALS AND METHODS.—Cultures of *E. amylovora* and an associated yellow bacterium were isolated from fire blight cankers collected from apple and pear orchards in Ohio. Single-colony isolates of both bacteria were selected and maintained on nutrient-glucose agar (per liter: Difco nutrient broth, 8 g; glucose, 10 g; agar, 20 g), and were stored at 4° C. Nutrient-glucose broth cultures grown at 28° for 24 hours on a reciprocal shaker were used as inoculum.

Cross-streak agar tests (2) were made on yeast-extract agar (per liter: yeast extract, 2 g; glucose, 10 g; agar, 20 g) at pH 6.2 or on yeast-extract agar prepared with 0.1 N phosphate buffer (KH_2PO_4 and K_2HPO_4) at pH 6.5.

Filtrates of bacterial cultures were prepared by passing 2-day-old yeast-extract broth cultures grown on a reciprocal shaker through bacteria-proof porcelain filter candles. Sterility of filtrates was tested by transfers to yeast-extract broth. Filtrates were tested for inhibition of *E. amylovora* by serial dilution in yeast-extract broth at pH 6.4 or in yeast-extract broth prepared with 0.1 M phosphate buffer at pH 6.8. One, 2, or 4 ml of filtrate was added, respectively, to 9, 8, or 6 ml of assay broth.

RESULTS.—*Isolation of bacteria from fire blight cankers.*—Cultures of *E. amylovora* and an associated yellow bacterium were obtained from fire blight cankers on Bartlett pear and the following varieties of apple: Jonathan, Rome Beauty, Baldwin, Yellow Transparent, Stayman, Red Delicious, Grimes, and McIntosh. Of several hundred colonies obtained, 40 isolates were selected for study. The associated bacterium was easily distinguished from *E. amylovora* by the yellow color of the colonies.

Characteristics of the bacterium associated with E. amylovora.—A study of 2 isolates revealed the associated bacterium to be a gram-positive, non-spore-forming, motile rod occurring in pairs or short chains. The colony color on nutrient agar is yellow, the color being most pronounced in recently isolated cultures and fading somewhat after repeated transfer. On trypticase-sugar agar the colony color is light yellow or almost white. Growth is spreading and the colony smooth and dull. The bacterium is aerobic or facultatively anaerobic. It produces acid from glucose or sucrose but does not ferment maltose or lactose. It does not produce gas. Gelatin is liquefied. Nitrate is not reduced. The bacterium is tentatively designated as a species of the genus *Bacterium*.

Inhibition of Erwinia amylovora in culture by Bacterium sp.—Thirty-five of the 40 isolates of *Bacterium* sp. were inhibitory to *E. amylovora* in agar-streak tests. Width of inhibition zones averaged 20 mm and ranged from 4 to 31 mm. Twenty of those isolates inhibitory to the pathogen on agar media produced culture filtrates that prevented growth of the pathogen when 1, 2, or 4 ml was added to 9, 8, or 6 ml, respectively, of yeast-extract broth. Inhibitory isolates in-

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creased the acidity of the agar adjacent to the streaks. There was a general correlation between the degree of acidity and the width of inhibition zones. Similarly, inhibitory filtrates usually were pH 4.0 or lower, whereas noninhibitory filtrates were usually pH 4.0 or higher. Because of the apparent relationship between acid production by *Bacterium* sp. and inhibition of *E. amylovora* in agar and liquid media, an attempt was made to determine whether inhibition was due to the change in reaction of the substrate or to some other effect, e.g. an antibiotic.

The growth response of *E. amylovora* to pH was determined by the use of nutrient-glucose agar, yeast-extract agar, potato-glucose agar (per liter: extract from 200 g potatoes; glucose, 10 g; agar, 20 g), nutrient-glucose broth, and yeast-extract broth. All media were prepared with 0.1 M citrate-phosphate buffer with the proportions of citric acid and Na_2HPO_4 adjusted to give media buffered from pH 3.6 to pH 5.0 at intervals of approximately 0.2 pH unit. Inhibition of each of 4 isolates of *E. amylovora* tested occurred at pH 4.2–4.4 or lower on all media. These data are in general agreement with those of other investigators (1, 4, 5).

The 35 inhibitory isolates of *Bacterium* sp. were tested for inhibition of *E. amylovora* on agar media buffered to maintain a pH level favorable for growth of the pathogen. On yeast-extract agar buffered at pH 6.5, only 1 isolate inhibited *E. amylovora*. All 35 isolates were inhibitory on unbuffered agar as in the previous experiment.

The inhibitory properties of culture filtrates from 6 isolates of *Bacterium* sp. were determined when cultures were grown in buffered or unbuffered yeast-extract broth and assayed in buffered or unbuffered broth. Culture filtrates from all 6 isolates behaved similarly. Four ml of filtrate from unbuffered cultures did not inhibit growth of *E. amylovora* when added to 6 ml of assay medium seeded with the pathogen and buffered at pH 6.8 (Table 1); likewise, filtrates from cultures buffered at pH 6.8 were not inhibitory when assayed in unbuffered or buffered media. Filtrates from unbuffered cultures of *Bacterium* sp., when added to unbuffered broth seeded with *E. amylovora*, prevented growth of the pathogen as in previous tests. These results indicated that inhibition of *E. amylovora* by *Bacterium* sp. was due to the increased acidity of the test media.

TABLE 1.—*Inhibition of Erwinia amylovora by culture filtrates from isolates of Bacterium sp. assayed in unbuffered and buffered media*[a]

	Inhibitory effect[b] in indicated assay medium and pH of assay medium plus culture filtrate			
	Unbuffered medium		Buffered medium	
Isolate	Inhibition	pH[c]	Inhibition	pH
22	+	4.2	—	6.5
24	+	4.3	—	6.6
28	+	4.2	—	6.5
34	+	4.3	—	6.4
36	+	4.2	—	6.7
70	+	4.4	—	6.6

[a] Reaction of culture filtrates was pH 3.6–3.9; that of unbuffered and buffered media was pH 6.4 and pH 6.8, respectively. Data were taken after 48 hours' incubation; data given were from 1 of 3 tests, all with similar results.

[b] + = Inhibition of *E. amylovora* by 4 ml of filtrate or less. — = No inhibition of *E. amylovora* by 4 ml of filtrate.

[c] pH values are those of media containing least amount of filtrate for inhibition.

The possibility that the inhibitory property of *Bacterium* sp. might be due to production of an antibiotic active only at acid conditions was considered. In such a case, inhibition could occur only at a pH level between that preventing growth of *E. amylovora* and that at which media were buffered in the above tests, i.e. between pH 4.2 and 6.8. Evidence against this possibility was obtained by measuring the pH of assay broth containing the least amount of culture filtrate required to inhibit growth of *E. amylovora*. The reaction of media in such cases was pH 4.2–4.4, which was below the minimum for growth of *E. amylovora* (Table 1).

In another experiment, filtrates from unbuffered cultures of isolate 22 of *Bacterium* sp. were added to assay broth seeded with *E. amylovora* and buffered from pH 3.6 to pH 5.0 at intervals of 0.2 pH unit with 0.1 M citrate-phosphate buffer. Inhibition occurred only in those tubes with media buffered below pH 4.2. These results are further evidence that the inhibitory action of *Bacterium* sp. on *E. amylovora* was due to the increased acidity of the substrate rather than to the production of an antibiotic.

Isolate 70 of *Bacterium* sp. behaved differently from the other 35 inhibitory isolates in cross-streak agar tests. This isolate produced sufficient acid to account for inhibition of *E. amylovora*, but it inhibited the pathogen on buffered as well as on unbuffered agar. Filtrates from buffered cultures, however, did not inhibit *E. amylovora*. It is possible that isolate 70 produced an antibiotic when growing on agar media.

None of several unidentified fungi isolated from fire blight cankers inhibited *E. amylovora* when tested on several different agar media.

DISCUSSION.—The biological role of *Bacterium* sp. in fire blight cankers is not known. Fire blight symptoms have been inhibited by the inoculation of apple or pear trees with mixtures of *E. amylovora* and antagonistic bacteria isolated from fire blight cankers (3, 6, unpublished results of Thomas et al). The failure of *E. amylovora* to cause disease in these experiments might have been due to acid production or to some other mechanism, e.g. antibiotic production, in the inoculated twig. It seems possible, however, that the failure of symptom development was due to the introduction of the pathogen into the host in an acid medium. In the present work, equal proportions of cultures of any of various isolates of *Bacterium* sp. and of cultures of *E. amylovora* gave mixtures with a pH level unfavorable for growth of *E. amylovora*.

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STUDIES ON THE BIOLOGY OF TILLETIA BROMI-TECTORUM[1]

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SUMMARY

Tilletia bromi-tectorum is widespread in the Pacific Northwest on the weedy grass *Bromus tectorum*. The bunt fungus changes the morphology of the host only slightly so that the disease is not readily apparent in stands of grass. Optimum temperature for spore germination was found to be approximately 5°, with most spore collections reaching maximum germination in about 14 days. Spore germination is similar to that of *T. caries*, with 9–12 primary sporidia usually being produced on a promycelium. In spore morphology, *T. bromi-tectorum* varies considerably so that it is difficult to place taxonomically. Some collections resemble *T. fusca* and others *T. guyotiana*; however, spores of *T. bromi-tectorum* are distinct from those of *T. caries* and *T. contraversa*. Cross inoculations of *T. bromi-tectorum* to wheat and to grasses other than *B. tectorum* resulted in no infection. Studies indicate that infection of *B. tectorum* usually takes place when the seedling is in the 1-leaf stage or later.

Bunt fungi that have been designated as *Tilletia caries* (DC.) Tul. have been reported on a number of wild and cultivated grasses (4, 5). This prompted intensive investigations of these fungi to determine their relation to bunt diseases on wheat. *Tilletia bromi-tectorum* J. Urries, which occurs on *Bromus tectorum* L., is by far the most widely distributed of the bunt fungi attacking grass in the Pacific Northwest. This weedy grass species, commonly known as downy brome or cheat, is omnipresent in both the wheat and range areas of the region and is a weed in or around nearly every wheat field. It is a winter annual with a growth habit similar to that of winter wheat and therefore might be suspected of harboring smut fungi pathogenic to wheat. The bunt fungus usually can be found wherever *B. tectorum* occurs; next to *T. caries* and *T. contraversa* Kühn, the causes of common and dwarf bunt of wheat, respectively, it is the most prevalent bunt pathogen in the area.

This fungus on *B. tectorum* has been studied intensively over a 3-year period as to geographical distribution, host range, spore morphology, spore germination, and taxonomic relationships. The initial findings are reported herein.

Geographical distribution.—*T. bromi-tectorum* was first described in 1943 by de Urries (3), who collected it on *B. tectorum* from only a restricted locality near Madrid, Spain, over a 10-year period. It was found in North America during 1945–1949 by Fischer (4), who identified it tentatively as *T. caries*. He reported the bunt disease to be widespread on *B. tectorum* in Colorado, Idaho, Montana, Nevada, Utah, and Washington. It also is known to be extensively distributed in Oregon and to occur in California. Its failure to be identified earlier probably was the result of its inconspicuous nature as compared with the prevalent *Ustilago bullata* Berk., the cause of head smut of *B. tectorum*. A bunted specimen of *B. tectorum* in the State College of Washington Phanerogamic Herbarium had been collected in Latah County, Idaho, in 1936. This collection antedates the first recognition of the disease in the field by some 10 years.

Symptoms.—The bunt on *B. tectorum* is characterized chiefly by its inconspicuous nature (Fig. 1). It modifies the growth habit of the host plant only slightly and therefore it is difficult to distinguish smutted from nonsmutted plants. De Urries (3) states that smutted plants are always dwarfed compared with nonsmutted ones, but observations on a large number of plants from the western United States have shown that smutted plants, although often slightly dwarfed, may be the same size or even larger than

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Cooperative investigations of the U. S. Department of Agriculture, Crops Research Division, Agricultural Research Service, and the Agricultural Experiment Stations of Idaho, Oregon, and Washington. Scientific Paper No. 1663, Washington Agricultural Experiment Stations, Pullman, Washington.

[2] Pathologist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Pullman, Washington.

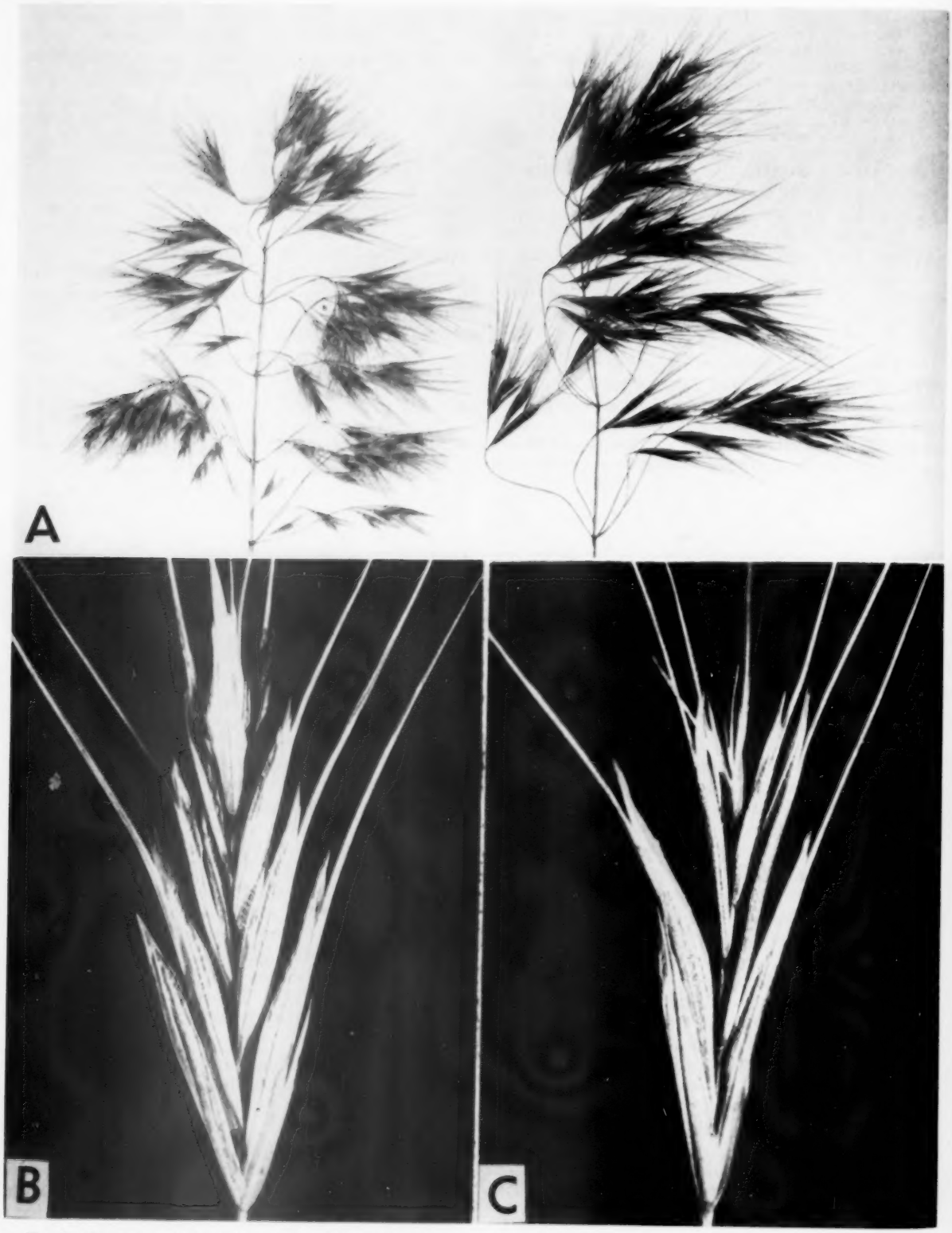

FIG. 1. Symptoms of bunt (caused by *Tilletia bromi-tecto um*) on *Bromus tectorum*. A) Bunted (left) and nonbunted panicles. B) Bunted spikelet. C) Nonbunted spikelet.

nonsmutted plants (Fig. 1, A). Often, the smutted panicle is slightly more compact than healthy ones. The spikelets of the smutted panicles may be broader than those of nonsmutted panicles because of the plump nature of the smut balls, as contrasted to the slender caryopsis (Fig. 1, B, C). Frequently the black smut balls can be seen through the thin lemma, or they may protrude from between the palea and lemma (Fig. 1, B).

According to de Urries (3), the smutted spikelets bear only 1 or 2 smut balls; the remaining flowers abort and contain no sori. In the United States, the number of florets that develop in each spikelet is the same in smutted and nonsmutted plants, and usually there are from 2 to 7 sori per spikelet (Fig. 1, B).

The spore mass of *T. bromi-tectorum* is dark brown to brownish black, with the latter color predominating. The shape of the smut balls ranges from long and narrow, similar in shape to the caryopsis of *B. tectorum*, to ovoid. The sori usually are from one-half to two-thirds the size of a seed of *B. tectorum*, as observed by de Urries.

As pointed out by Fischer (4), the bunt fungus on *B. tectorum* superficially resembles *Ustilago bullata*, which is also widespread on this host. They can be readily distinguished, however, since the latter involves the palea and lemma whereas the former involves only the caryopsis, and leaves the floral bracts intact.

Simultaneous occurrence of bunt and head smut on Bromus tectorum.—Several plants affected by both head smut and bunt have been collected. This fact suggests that their simultaneous occurrence in the same plant may be frequent, since these smuts often are coexistent in stands of *B. tectorum*. They may occupy the same panicle and in some cases the same spikelet. One panicle was examined in detail to determine the pattern of occurrence of the 2 diseases in individual spikelets (Fig. 2). Of a total of 44 spikelets in the panicle, 25 had bunt, 15 had head smut, and 4 had both diseases. The bunt predominated at the top and bottom of the panicle, whereas head smut predominated in the middle section. The finding of only 4 spikelets having both smuts may indicate some competition between the parasitic mycelia of the 2 species of fungi.

Spore mounts from individual florets sometimes contained spores of both disease organisms, but it was impossible to determine whether the Ustilago spores originated from within the sorus of the bunt fungus or were merely contaminations from sori of *U. bullata* in the same panicle.

Spore germination.—De Urries (3) obtained spore germination in both distilled water and tap water to which a small portion of soil had been added. Germination began at the end of 10–12 days incubation at a temperature range of 5° to 12°C. No germination occurred at 20°.

In the present investigations, 37 collections of *T. bromi-tectorum* from *B. tectorum* made during the summer of 1954 were studied for germination characteristics during the following winter and spring. These collections were made from widely scattered locations in Washington, Oregon, Idaho, Montana, and Utah. The spores were surface sterilized by soaking for 1 minute in 5 per cent solution of Clorox (5.25% sodium hypochlorite), washed in sterile distilled water, and plated on 3 per cent agar in culture dishes. The dishes were then divided into 4 groups and placed in incubating refrigerators at 2°, 5°, 10°, and 15°C.

The optimum germination temperature for the spores of all collections was 5°, although some germination occurred at all 4 temperatures. Maximum germination occurred also at 2° but at a slower rate than at 5°. At 10°, the spores of 6 of the 37 collections germinated but at a much slower rate than at the lower temperatures; of these 6, only 2 collections showed more than small percentages of germination. The spores of only 1 collection reached maximum germination at 10°, and this collection also germinated at 15°. It seems evident that spore germination in the great majority of the collections occurs only at relatively low temperatures (2°–5°).

A wide range in rate of spore germination was

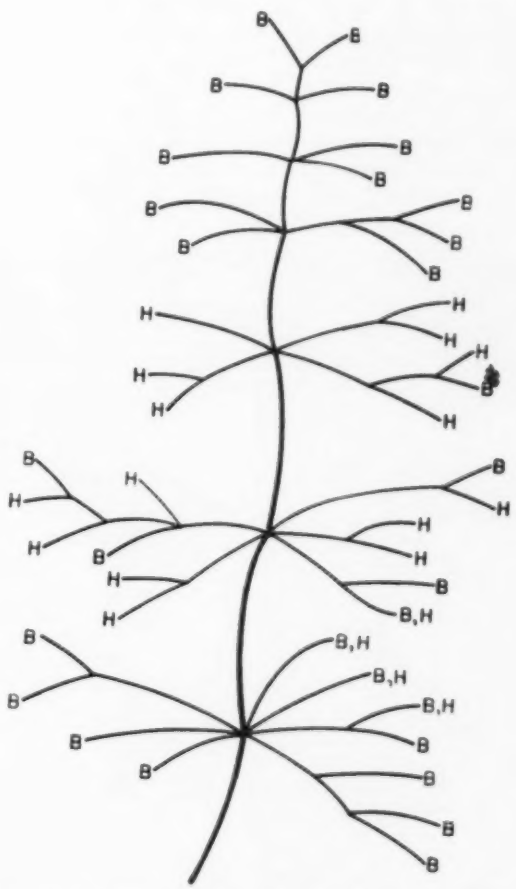

Fig. 2. A diagram of a panicle of *Bromus tectorum* infested with the bunt and head smut pathogens, showing the relative location of spikelets with bunt (B), with head smut (H), and with both diseases (B, H).

shown by the different collections. At 5°, 21 of the 38 collections began to germinate in 8–10 days and reached a maximum in approximately 14 days. Eleven of the collections began germinating after 14 days' incubation and reached maximum germination in about 20 days. Only 5 collections took 20 days or longer to germinate.

The rate of germination of *T. bromi-tectorum* was compared with that of other species of *Tilletia* whose optimum temperature for germination is about 5°. Nineteen collections identified as *T. guyotiana* Har. from various weedy annual or winter annual bromes (from the section *Bromium* of the genus Bromus) and 11 collections of *T. fusca* Ell. & Ev. from annual fescues were somewhat slower in germination than most collections of *T. bromi-tectorum*. These usually began to germinate in 14–20 days and reached maximum in about 30 days. At 5°, the spores of *T. elymi* Diet. & Holw., *T. cerebrina* Ell. & Ev., and *T. contraversa* germinated much slower than did those of *T. bromi-tectorum*. These 3 species required from 20 days to more than a month. It appears, therefore, that spores of *T. bromi-tectorum* germinate faster than do those of most other species of smut fungi occurring on grasses in the Pacific Northwest; however, the germination is somewhat slower than that of *T. caries*, which germinates in 3–5 days.

Spore germination of *T. bromi-tectorum* is similar to that of *T. caries* and is illustrated in Figure 3, A. Under optimum conditions for spore germination, the promycelium is relatively short and usually bears from 9 to 12 primary sporidia. Under suboptimal conditions, however, the promycelia elongate considerably before primary sporidia are formed. Under such conditions, only 6–8 primary sporidia are formed on each promycelium. The primary sporidia fuse in pairs, and secondary sporidia are formed as in *T. caries*. Primary sporidia were isolated and grown in culture, where secondary sporidia were produced in abundance. As a result, the cultures had a powdery appearance.

Spore morphology.—The spore morphology of 35 collections of *T. bromi-tectorum* was studied in detail. These collections were from widely separated locations throughout the western United States. A portion of the type collection of *T. bromi-tectorum* from Madrid also was examined.

For microscopic examination, the spores were mounted in Shear's mounting medium by means of the double-cover glass technique as described by Chupp (2). From 5 to 10 spores taken at random on a slide were measured for diameter, size of areolae, depth of reticulations, and thickness of sheath.

The spores varied in shape from globose to ovoid; many collections were predominantly one or the other. The average diameter of spores from individual collections ranged from 21.3 to 24.1 μ; that of individual spores varied from 16.6 to 28.2 μ. The average diameter of all spores measured was 22.8 μ. Of 307 spores measured, 258 were between 21.1 and 25.0 μ. These spore dimensions agree with those reported by de Urries (3) and with those of portions of the type specimen examined.

Sterile cells were present in all collections studied. Such cells were hyaline, globose to ovoid, and smooth.

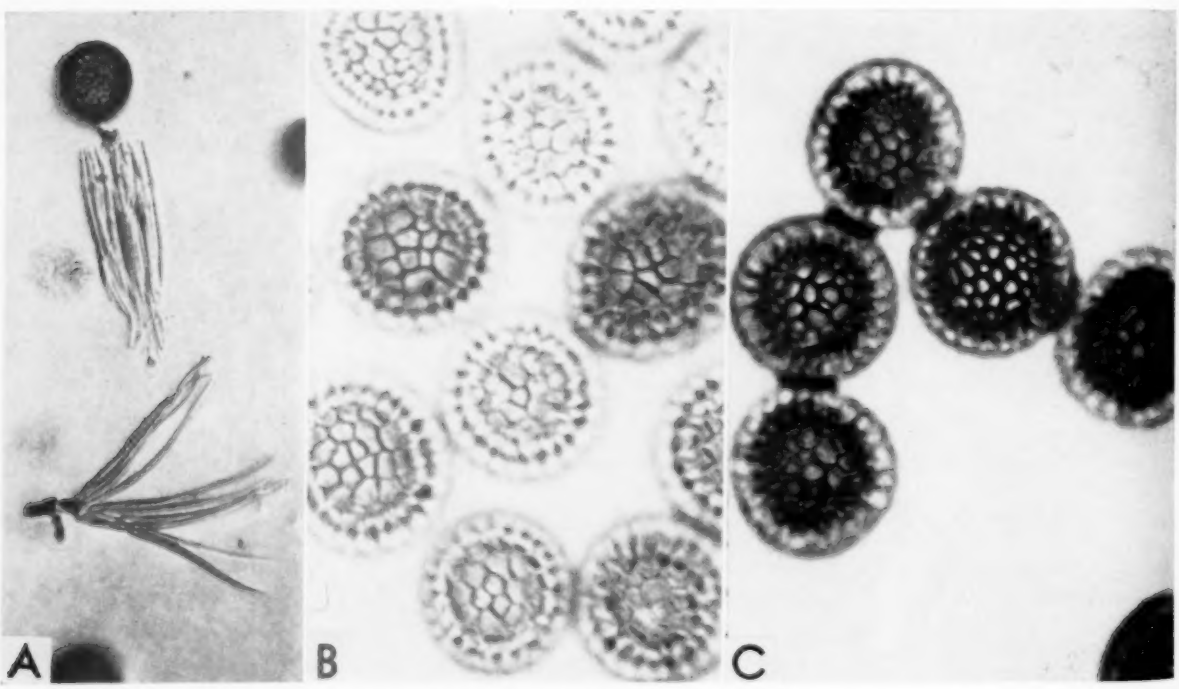

Fig. 3. A) Germinating spores of *Tilletia bromi-tectorum*. B,C) Spores of *T. bromi-tectorum*, showing differences between spore morphology and pigmentation of 2 collections of spores.

In diameter they ranged from 10.0 to 20.0 μ, but were usually between 13.0 and 18.0 μ.

Areolae varied greatly in size and shape both in comparisons of spores of a single collection and of spores of different collections (Fig. 3, B, C). In these collections, the areolae ranged from less than 1 μ to 10 μ in their greatest diameter. They were oval, rectangular, or 5 or 6 sided. The depth of reticulations ranged from 1.0 to 3.3 μ, with the great majority falling between 1.5 and 2.5 μ. The depth of reticulations on a few spores was too shallow to measure. Occasional cerebriform spores were seen.

The hyaline sheath was usually very thin, projecting less than 1 μ beyond the reticulum.

The color of the spores from the various collections ranged from yellow through various shades of brown to dark brown, and it varied both between and within collections (Fig. 3, B, C). Frequently spores from the same sorus ranged from yellow to dark brown (Fig. 3, B). This variation appears to be correlated with depth of areolae: spores having relatively shallow and numerous reticulations generally are darker (Fig. 3, C) than those with large areolae and deep reticulations (Fig. 3, B). Some spores are so dark that they are opaque, and these invariably have very shallow reticulations. Between these 2 extremes lies an intergrading series of spores with characteristics of both.

Taxonomic relationships.—According to de Urries (3), *T. bromi-tectorum* in Spain appears to be closely related to *T. guyotiana*. Certain collections on *B. tectorum* from the western United States were assigned to *T. caries* by Fischer (4). Siang (8) identified collections from *B. tectorum* as *T. guyotiana*. This indicates that the species on *B. tectorum* resemble several species of *Tilletia* in spore morphology. To clarify this point, spores of a larger number of collections from *B. tectorum*, including those studied by de Urries, Fischer, and Siang, were compared with the descriptions and type specimens of *T. fusca* and *T. guyotiana*. Collections with dark spores and fine reticulations resemble *T. fusca;* those with spores light in color and with relatively large areolae might be classified as *T. guyotiana*, although the spore mass is darker than those typical of the latter species.

It appears, therefore, that in spore morphology, *T. bromi-tectorum* provides an intergrading series between *T. fusca* and *T. guyotiana*. In contrast, the spores of *T. bromi-tectorum* appear to be quite distinct from those of *T. caries* and *T. contraversa*, since they average several microns larger than do those of *T. caries* and *T. contraversa* and do not have a prominent sheath as do spores of *T. contraversa*. In addition, spores of *T. bromi-tectorum* are much darker in color and have deeper and more prominent reticulations than do those of the other 2 species.

Host range.—*T. bromi-tectorum* has been previously reported only on *B. tectorum*, but 1 collection used in this study was from *B. rigidus* Roth, and another was from *B. sterilis* L. All 3 of these grass species are in the subsection (*Eubromus*) of the genus *Bromus* and are, therefore, presumed to be closely related.

One of the main objectives of the study of *T. bromi-tectorum* was to determine if it played any role in the wheat bunt problem in the Pacific Northwest. The 10 common bunt differential wheat varieties (7) were inoculated by several techniques that are described below. Parallel inoculations of the original host, *B. tectorum*, and of other *Bromus* species (*B. brizaeformis* Fisch. & Mey., *B. commutatus Schrad.*, *B. japonicus* Thunb., *B. mollis* L., *B. marginatus* Nees, and *B. racemosus* L.) were made.

No bunt developed on the wheat varieties or on species of *Bromus* other than *B. tectorum;* however, all of the 14 collections of *B. tectorum* inoculated developed bunt, with infection percentages ranging from 3 to 24.

Infection studies.—Several methods of inoculation were tried in an attempt to determine how and when infection takes place. Since the spores germinate in a relatively short time (8–12 days at 5°C), it seemed possible that spores on the seed might germinate and invade the host by seedling infection in a manner similar to that of spores of *T. caries*. Seed of *B. tectorum* and other grasses was inoculated by placing the spores in a water suspension and subjecting the seed to partial vacuum. The inoculated seed was then allowed to germinate at various temperatures. Only 1 row from seed inoculated in this manner produced any bunt (1%). This lot had been subjected to low temperatures out-of-doors for several weeks after inoculation. Although it is possible to obtain infection by this method, it seems likely that seed-borne spores are not the primary source of inoculum in nature.

Other methods tried have been used successfully with dwarf bunt of wheat (6). These methods consisted of germinating the spores on agar in petri dishes and placing the germinating spores on the seed by various means. Subsequently the seed was allowed to germinate at various temperatures. Results obtained with these methods have been very erratic and largely unsuccessful, although up to 17 per cent bunt was obtained in some cases.

A third method, which appears promising, has been used with considerable success with dwarf bunt of wheat (1). This consisted of spraying the soil surface with a suspension of spores following seeding. Up to 24 per cent bunt on *B. tectorum* has been obtained by this method.

Up to 56 per cent smut on *B. tectorum* was obtained by the following method. Seedlings growing in 4-in. pots were allowed to develop to the 1-leaf stage, at which time they were sprayed with a water suspension of germinating spores and then incubated for some 10 days at 5 or 10° before transplanting to the field.

The success of the last 2 methods of inoculation described above suggests that infection by *T. bromi-tectorum* occurs after seedling emergence. In nature, the seeds of *B. tectorum* germinate with the first rains in late summer or early autumn, at which time the temperature range probably is above optimum for spore germination. By the time the temperature is sufficiently low to stimulate spore germination, the seedlings

probably are in the 1-leaf stage or older. Apparently, infection occurs at this or a later stage of development in a manner not yet determined.

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UNUSUAL FUNGI ASSOCIATED WITH DECAY IN SOME FOREST TREES IN COLORADO[1]

Ross W. Davidson and Thomas E. Hinds[2]

SUMMARY

Studies of heart rot in mature timber at high elevations have revealed several wood-decaying fungi not previously reported as infecting living trees. A white butt rot fungus, *Corticium radiosum*, was most frequently isolated from subalpine fir. A brown pocket rot beneath basal wounds in this same species is caused by *Helicobasidium corticioides*. The most frequently isolated fungus from main stem decay in trembling aspen was identified as *Cryptochaete polygonia*. *Peniophora luna*, which was previously reported as the cause of a top or trunk rot in lodgepole pine (from identification of cultures), fruits abundantly in the areas where this tree grows.

In the course of economic studies on decay in spruce-fir timber growing at high elevations in Colorado, a number of common fungi, which later proved of particular interest in studies of decay in living trees, were encountered. Basidiospore cultures were obtained from as many of these fungi as possible—especially species not previously known to cause decay in heartwood of living trees.

Test tubes, as illustrated in Figure 1, were used almost exclusively to obtain basidiospore cultures. This test tube method is similar to the one commonly used to make spore prints directly on culture media in petri dishes but is much more efficient for field use.

The fungi involved were those fruiting on decaying logs, stumps, and dead tops of Engelmann spruce (*Picea engelmannii* Perry), subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), lodgepole pine (*Pinus contorta* Dougl.), and quaking aspen (*Populus tremuloides* Michx.). This study was made at elevations ranging from about 9,000 ft. to timber line at 11,500 ft.

A BROWN POCKET ROT ASSOCIATED WITH HELICOBASIDIUM CORTICIOIDES.—Among the fungi commonly encountered on dead wood was one that formed white to light gray, thin, crustose layers on dead decorticated coniferous logs and that was always associated with a brown pocket rot (Fig. 2, A). The compound microscope showed these crustose layers to contain coiled, spiraled basidia protruding from a loosely arranged layer of hyaline hyphae. Specimens from which basidiospore cultures were obtained were submitted to Dr. R. J. Bandoni for identification. He described the fungus as a new species, *Helicobasidium corticioides*[3]. Spore prints were obtained directly on culture media in test tubes and on glass slides. Spores usually were not deposited in great abundance but could sometimes be detected as a very sparse white layer. The spores were large, hyaline, and broadly ovoid,

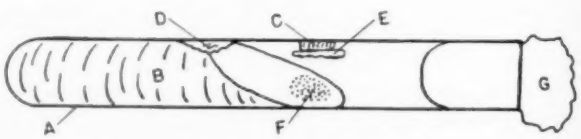

FIG. 1. Diagram of test tube method of obtaining basidiospore cultures of hymenomycetes. A) Test tube. B) Culture-medium slat. C) A glob of culture medium removed from point D) serves both to hold the portion of the sporophore to the inside of the test tube and to keep it moist. E) Section of sporophore with hymenium side directed toward the slanted surface of the culture medium. During sporulation the tubes were stored in such a position that the slanted surface of the medium was directly below the hymenial surface of the sporophore (10-36 hours usually sufficient time). G) Cotton plug.

[1] Accepted for publication November 12, 1957.

[2] Forest Pathologist and Forestry Aid (Research), respectively, the Rocky Mountain Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture, with central headquarters at Fort Collins, Colorado, in cooperation with Colorado State University.

[3] Bandoni, R. J. 1955. A new species of Helicobasidium. Mycologia 47: 918-919.

with a prominent apiculus. Germination was not profuse, but short narrow germ tubes were present on some spores in 2–3 days. Spore prints on media (malt or cornmeal agar) did not show any visible growth for about 4 weeks, even when the spores were known to have germinated in 3 days. Thereafter a white sparse mycelial growth became visible.

Cultures grew very slowly and were pure white.

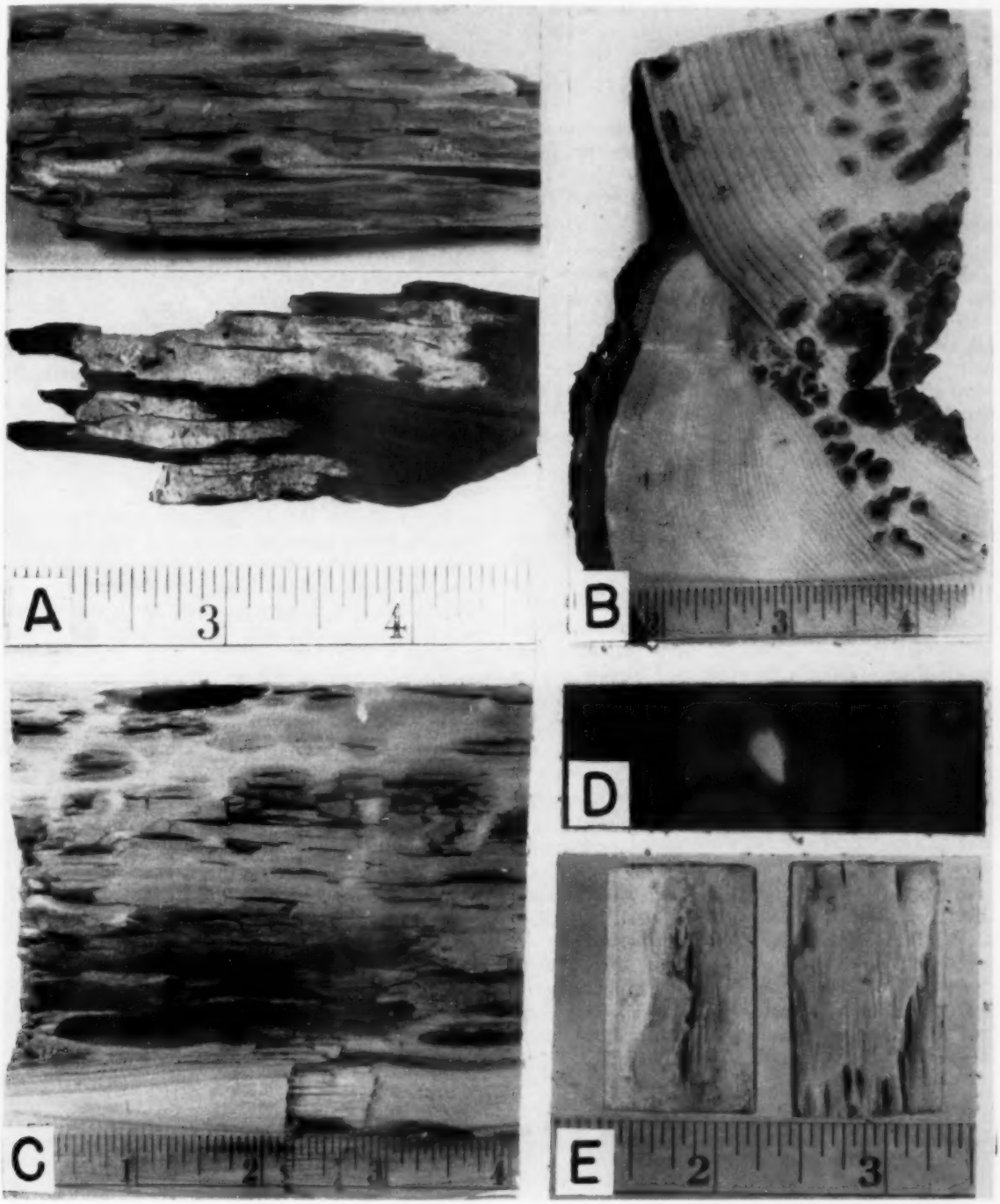

FIG. 2. *Helicobasidium corticioides* and brown pocket rot. A) Corticium-like fruiting and associated rot in dead wood. B) Cross section of brown pocket rot associated with a basal wound in living subalpine fir. C) Longitudinal section through the same basal rot. D) Pure basidiospore culture of *H. corticioides* grown on malt-agar medium at a temperature of 65° F, 6 weeks' growth, natural size. E) Brown pocket rot in sapwood blocks of subalpine fir on which pure cultures of Helicobasidium were grown for 12 months at 65° F.

rather thin, and cottony (Fig. 2, D). Attempts to grow this fungus at Beltsville, Maryland,[4] on 3 differential agar media (malt, malt plus 0.5% tannic acid, and malt plus 0.5% gallic acid)[5] at room temperature (about 75°F) resulted in only a trace of growth in 2–3 weeks. Reaction of the fungus on the tannic or gallic acid media, used to test for oxidases, was uncertain because of slow growth and formation of a dark diffusion zone when transferred to plain malt agar.

Cultures seemed to grow best at temperatures of 60°–70°F, but even under the most favorable conditions the fungus often makes no growth when transferred to new tubes of culture medium. Usually it has been necessary to make a number of transfers in order to get 1 or 2 that will show some new growth.

During an investigation of decays in living subalpine fir in the summer of 1953, a brown pocket rot occasionally was found back of basal wounds (Fig. 2, B, C). In 1 area where fire wounds were common, this type of rot was present at the base of 6 trees. Of a total of 196 trees cut and dissected, 11 contained this decay behind basal wounds. The decay never extended more than about 1 ft. beyond the wounded area and, therefore, did not cause much volume loss.

Isolations from the decay usually yielded only secondary fungi; however, a very slow-growing white mycelium grew from 2 of the infections. After about 4 months, these 2 cultures were examined and found to contain coiled basidia similar to those collected on decorticated logs earlier. In general appearance the cultures were very similar to those of *Helicobasidium corticioides* Bandoni obtained from basidiospores. Transfers were difficult to grow, and almost always a dark zone developed under the transferred mycelium.

The presence of a brown pocket rot consistently associated with the Helicobasidium sporophores, the similarity of the brown pocket rot associated with basal wounds, and the fact that Helicobasidium was the only fungus isolated that was considered at all likely to cause decay strongly suggested that the rot was actually caused by the Helicobasidium.

Pure culture of *H. corticioides* were grown on autoclaved wood blocks placed on moist sterilized soil in 6-oz. bottles. This method is similar to the one described by Leutrize.[6] After 12 months at a temperature of 65°F, the blocks were removed and found to have brown pocket rot similar to that in wood on which *H. corticioides* has been found under natural conditions (Fig. 2, E).

[4] The growth studies were made by Mrs. Frances Lombard, Pathologist, Forest Disease Research Laboratory, Plant Industry Station, Beltsville, Maryland.

[5] Davidson, R. W., W. A. Campbell, and D. B. Vaughan. 1942. Fungi causing decay of living oaks in the eastern United States and their cultural identification. U. S. Dept. Agr. Tech. Bull. 785, 65 p.

[6] Leutritz, J., Jr., 1939. Acceleration of toximetric tests of wood preservatives by the use of soil as a medium. Phytopathology 39: 901-903.

OTHER COMMON FUNGI ASSOCIATED WITH DECAY IN LIVING TREES.[7]—*Peniophora luna Rom.*—In 1950,[8] Hornibrook published the results of a study of decay in 3 Rocky Mountain conifers. In this report, he mentions that *P. luna* entered and caused a brown rot in trunks of lodgepole pine. This fungus was at that time identified from sporophores that developed in cultures in the laboratory. In studies of slash fungi mentioned in this paper, it was found that *P. luna* is one of the common fungi fruiting on lodgepole pine logs in the woods. It has also been observed on dead branch stubs of living trees.

Corticium radiosum (Fr.) Fr.—In the recent study of decay in living subalpine fir, the fungus isolated most frequently from a white butt rot did not appear to be any of the fungi previously reported as causing decay in living trees. A comparison of the cultures isolated from the decay in living trees with basidiospore cultures of *C. radiosum* suggests that they are the same. At present we have no reports of *C. radiosum* ever having been isolated from living trees. It was isolated from 16 trees in the study conducted in the central Rockies. The fungus commonly fruits on dead wood on the ground in the spruce-fir forests at high elevations.

Cryptochaete polygonia (Pers. ex Fr.) Karst.—A preliminary study of decay in aspen in the central Rockies indicates that Cryptochaete may be the most common fungus causing trunk and top rot in this species.[9] Fruiting bodies are common on dead trees lying on the ground and have been observed on a dead branch stub on a living tree. Identifications were made by isolating the fungus and identifying the cultures. It causes a white rot, similar to early stages of decay caused in aspen by *Fomes igniarius* var. *populinus* (Neuman) Campbell.

Although Cryptochaete was present in a greater number of cases (total of 113) than was *F. igniarius* var. *populinus* (total of 102), the latter fungus causes much more loss by volume in stands 100–150 years of age then does the former.

The cultural characteristics of these fungi form the basis for identification from decay specimens in the absence of fruiting bodies; data on these characteristics will be published later.

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[7] Identifications of the sporophores used in this study were made by Dr. Paul L. Lentz, Mycologist, National Fungus Collections, U. S. Dept. Agr., Beltsville, Maryland.

[8] Hornibrook, E. M. 1950. Estimating defect in mature and overmature stands of three Rocky Mountain conifers. J. Forestry 48: 408-417.

[9] No published report indicating that *Cryptochaete polygonia* causes decay in living trees has been found; however, an interim report by R. L. Black and R. J. Bourchier on decay in aspen in the Manitoulin areas of Ontario, July, 1952, listed 6 isolations of this fungus. Another interim report by R. L. Black and P. J. Kristopovich on decay in aspen in Manitoba and eastern Saskatchewan, April, 1954, lists 124 isolations in this fungus. These reports are from Forest Biology Laboratories, 144 Front Street, West Toronto, Ontario.

EFFECTS OF A MERCURY FUNGICIDE AND AN INSECTICIDE ON GERMINATION, STAND, AND YIELD OF SOUND AND DAMAGED SEED WHEAT[1]

C. W. Roane and T. M. Starling[2]

SUMMARY

In 1954, Virginia seedsmen reported many instances of mercury toxicity or injury to wheat seed that resulted in a decline in germinability of the lots examined. The decline was from an average original germination of 92 per cent to one of 76 per cent. Seed that declined in viability was either withdrawn from the market or relabeled with reference to percentage germination and sold at a reduced price. The injury by a mercury fungicide to seed wheat reported in this paper resulted from penetration of the excessively cracked and chipped pericarp that normally protects the embryo, coupled with prolonged storage after treatment. In experiments with seed lots injured by a mercury fungicide, sound seeds germinated better and yielded more grain than did either cracked or chipped seeds, and chipped seeds yielded significantly less than did either sound or cracked seeds. Thus, the fungicide, Ceresan M, proved to be severely phytotoxic to the chipped seeds, slightly toxic to the cracked seeds, and nontoxic to the sound seeds. The application of an insecticide, Pyrenone, to seed wheat had no apparent effect on seed germination or yield of grain. It is recommended that the physical condition of the pericarp covering the embryo be determined for all lots of seed wheat to be used in seed-treatment experiments and that seedsmen treat and store only seed wheat that contains no more than a very small percentage of damaged kernels.

In 1954, the Virginia Department of Agriculture reported that a large number of wheat seed lots that had been certified as germinating 90 per cent or more were withdrawn from the market because they failed to germinate as labeled. Between the time the seed lots were certified and the time they were distributed to seed dealers, germination declined from an average of 92 per cent to an average of 76 per cent for the several seed lots involved. All that declined in germinability had been treated with mercury compounds. The seed lots involved had been treated by several seed distributors. According to Jones et al (9), high temperatures prior to harvest may have made the pericarp susceptible to injury during harvest. It was concluded that this injury or opening exposed the embryo to mercury damage during storage under the high temperatures that persisted in Virginia in 1954. A few samples were injured in 1955; in 1956, however, seedsmen again complained of large-scale reduction in germination of mercury-treated seed.

Samples of Vahart wheat (C. I. 12537)[3] were obtained from one of the companies that had withdrawn seed lots from the market because of a decline in germination. Samples were obtained that had been treated commercially with Pyrenone,[4] with Ceresan M,[5] and with a combination of these, and seed that had received no treatment. The 4 samples were portions of a lot originating from the same farm. These samples were studied to determine proportions of damaged and sound kernels, the effects of the fungicide and insecticide on viability of sound and damaged kernels, and the effects of injuries on yield. The results of these studies are reported in this paper.

REVIEW OF LITERATURE.—Phytotoxicity of seed-treatment chemicals has been associated with seed coat injury at least since 1872, when, according to Hurd (8), Nobbe (14) reported that machine-threshed seed was injured more by copper sulphate than was hand-threshed seed because damage done to the seed coats by the machine allowed the copper sulphate to penetrate to the embryo. Nobbe also pointed out that the drier and more brittle the grain, the greater the threshing injury. Falke (6) noted the effects of copper sulphate on seed with broken seed coats and found that seed produced in a dry year was more subject to treatment injury than was seed produced in a normal year. Volkart (19) reported that breaks in the seed coat exposing the embryo allowed damage by copper sulphate whereas those exposing endosperm did not allow appreciable damage. This was confirmed later by Woolman (21), Walldén (20), and Hurd (8). Hurd concluded that damage done to seed wheat by copper sulphate could be predicted by the physical condition of the seed. Perfect seed coats also were considered to be an absolute protection against damage from short exposures to strong formaldehyde. Zundel (22) reported similar results and, like Hurd, pointed out that damage increased as the cylinder speed of the threshing machine increased.

Phytotoxicity of mercury compounds to treated wheat seed has been recognized and described by several workers (1, 2, 4, 9, 12, 16). Crosier (2) apparently was the first to describe mercury injury of wheat. He pointed out that the injury is expressed by production of stubby roots and coleoptiles and that only seeds whose embryos were exposed as the result of cracked and chipped pericarps were injured by mercury compounds. Dillon Weston and Booer (4) apparently discovered the same relationship without knowl-

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[3] The C. I. number is the cereal investigation accession number of the United States Department of Agriculture.

[4] Pyrenone, an insecticide, contains as active ingredients 1.1 per cent piperonyl butoxide and 0.08 per cent pyrethrins.

[5] Ceresan M contains 7.7 per cent *N*-(ethylmercuri)-*p*-toluenesulfonanilide.

edge of Crosier's report. Porter (16) later substantiated these findings and gave a detailed description of the injury, including polyploid nuclei in the stubby sprouts. Brett and Dillon Weston (1) found that mercury-treated seed declined in viability after prolonged storage if the initial moisture content was greater than 15 per cent. They illustrated and described stubby coleoptiles and roots but did not refer to damaged pericarps. Koehler (10) studied the effects of mercury treatment on yield of wheat after various storage periods. He reported suppression of yield after prolonged storage but did not take into consideration the proportion of seeds with breaks in the pericarp. Machacek and Wallace (12) found that top grades of wheat that contained more plump kernels than did intermediate grades were more subject to the threshing damage and, consequently, were more subject to mercury injury than were lots of intermediate grades. More recently, Midyette (13), Crosier and Midyette (3), and Jones et al (9) have reported an uncommonly severe occurrence of mercury injury coupled with pericarp damage in seed stocks from Virginia.

Overland and Nelson (15) reported that slurry treatment with copper carbonate reduced seed germination of 5 varieties of wheat when compared with treatment with copper carbonate dust and with no treatment. They made no reference to previous work on phytotoxicity of fungicides to damaged seed. Purdy and Holton (18) and Purdy (17) have reported inconsistent results with phytotoxicity studies on seed wheat; they attributed these to differences in soil moisture and temperature. In 1954, they reported the seed of Idaed and Federation wheat as sensitive to treatment; in 1955, however, Purdy considered Idaed seed tolerant and Federation seed sensitive to mercury treatments. In neither case was the condition of the seed considered. Koehler and Bever (11) obtained differences in seed germination that resulted from a high dosage of fungicide, a long storage time, and a high moisture content. The differences in phytotoxicity were not in proportion to the volatility of the compounds used. They reported that seed of Vigo wheat was injured more than that of Pawnee, but they did not determine whether the 2 varieties differed in pericarp damage.

TABLE 1.—*Accumulated percentage germination of kernels from the 4 treatments after incubation for various periods in Arasan-treated and untreated paper towel ragdolls*[a]

Seed treatment	Ragdoll treatment	Percentage of kernels germinated after indicated number of days			
		4	7	10	15
None	None	89.4	96.2	96.2	96.2
	Arasan	85.4	94.6	94.6	94.8
Pyrenone	None	91.8	95.8	95.8	95.8
	Arasan	87.0	96.2	96.4	96.4
Ceresan M	None	82.0	94.2	95.3	95.4
	Arasan	80.2	82.2	94.0	95.0
Ceresan M + Pyrenone	None	80.2	90.6	91.8	91.8
	Arasan	72.2	92.0	93.2	93.6

[a] Average of 5 replicates, each consisting of 100 kernels.

TABLE 2.—*Accumulated percentage of germination of sound, cracked, and chipped kernels after incubation for various periods in rolled paper towels*[a]

Seed treatment	Seed condition	Percentage of kernels germinated after indicated number of days		
		4	7	10
None	Sound	46.7	98.0	99.5
	Cracked	57.2	96.7	98.0
	Chipped	28.2	69.0	84.2
Pyrenone	Sound	48.0	93.7	95.2
	Cracked	47.0	94.2	96.0
	Chipped	41.5	82.2	86.7
Ceresan M	Sound	40.5	87.2	97.2
	Cracked	30.7	90.0	95.2
	Chipped	21.5	52.5	73.2
Ceresan M + Pyrenone	Sound	47.5	94.8	98.8
	Cracked	22.7	79.5	92.2
	Chipped	18.5	50.2	72.5

[a] Average of 6 replicates, each consisting of 100 kernels.

Hsi (7) studied the effect of 4 fungicides containing mercury at 4 rates of application and 2 storage periods on germination and seedling injury of 2 varieties of wheat. He concluded that mercury injury to wheat is influenced by kind and dosage of fungicide, duration of storage after treatment, and inherent differences in wheat. Hsi failed to determine the physical condition of the pericarp even though he was aware of Crosier and Midyette's report.

MATERIALS AND METHODS.—The seed used for the experiments reported herein was obtained from the Southern States Cooperative, Richmond, Virginia, and came from lot number 3052AD of Vahart wheat. The samples provided were an untreated sample and 3 samples treated as follows: 1) with Pyrenone, 1⅛ oz./bushel; 2) with Ceresan M applied as a slurry, ½ oz./bushel; and 3) with Pyrenone + Ceresan M, 1⅛ and ½ oz./bushel, respectively. These treatments were applied in August, 1954, and the 4 seed samples had been stored in a commercial warehouse until late October, when the writers obtained the seed. During the period of storage the samples were exposed to a brief period of temperatures in excess of 100°F. Separations of each sample into groups of sound seeds, of those with cracks in the pericarp over the embryo, and of those with chips or missing parts in the pericarp that exposed the embryo were made with the aid of a 20× dissecting microscope.

Germination tests were carried out in paper-towel "ragdolls." One hundred seeds to be germinated were rolled into folded-type paper towels that were flattened before they were used. The ragdolls were held in a rolled position by rubber bands. Five or 6 groups of ragdolls, each group a replicate, were bound together and placed upright in a dish. After enough water was added to the dish to moisten the towels, the dish and towels were covered with a bell jar for the duration of the germination period. Yield determina-

tions were made with 6 replications of single-row plots, the rows being 10 ft. long and 1 ft. apart; seeding was at the rate of 10 g of seed per row. The yield data were analyzed statistically. The single-row plots were not entirely suitable for yield determinations, but plot size was determined by the seed available, which was limited by the tedious task of separation.

RESULTS.—*Proportion of sound and damaged seed in the 4 treatments.*—Throughout this study, seeds with no visible damage were considered sound. Damaged seeds were those with breaks in the pericarp over the embryo only. Two types of damage were noted. In some cases, there was merely a fissure in the pericarp; in others, small pieces of the pericarp were missing. Hereafter, these types of damage are referred to as cracked and chipped, respectively. About 33 per cent of the kernels were sound, 34 per cent cracked, and 33 per cent chipped, a total of 67 per cent damaged kernels. The seedlots were remarkably uniformly divided among the 3 conditions.

Germination of seed in ragdolls.—Preliminary tests indicated that paper-towel ragdolls would be satisfactory for the determination of the viability of seed that had been subjected to the various treatments; however, saprophytic fungi occurring in the towels occasionally interfered with the results. Therefore, it seemed necessary to treat the paper towels with a fungicide to prevent the growth of saprophytic fungi. In an effort to solve this problem, a comparison was made between seeds germinated in towels treated with Arasan[6] and those in untreated towels. Germinated seeds were counted and removed from the ragdolls after 4, 7, 10, and 15 days. After an incubation period of 4 days, most seeds were observed to be germinating normally but some had stubby sprouts, evidence of mercury injury; however, all that sprouted were counted, with no attempt to separate normal from abnormal seedlings.

After 4 days, seeds in untreated towels had germinated better than those in Arasan-treated towels, but after 7, 10, and 15 days, there was little difference between counts from treated and untreated towels (Table 1). After 10 days, more than 90 per cent of the seeds of all treatments had germinated. Fewer fungi developed in the Arasan-treated towels; therefore, Arasan was used to treat the ragdolls for the control of saprophytic fungi.

Germination of sound and damaged seed.—Separation of sound, cracked, and chipped kernels in each treatment were made and the seeds were germinated in Arasan-treated paper-towel ragdolls. The experiment was performed in November, 1954, 3 months after the seed were commercially treated. When compared with sound kernels, chipped kernels germinated poorly and slowly. Cracked kernels germinated somewhat slower than did sound ones but were not as subject to killing as were chipped kernels. Ceresan M retarded the germination and killed some chipped kernels. Pyrenone produced no measurable effects. Although counts were made after 21 days, no differences were observed and a count after 10 days was satisfactory (Table 2).

[6] Arasan contains 50 per cent bis(dimethylthiocarbamoyl) disulfide (=thiram) as the active ingredient.

TABLE 3.—*Estimated stand on April 28, 1956, of wheat grown from sound, cracked, and chipped seed treated with combinations of Pyrenone and Ceresan M* [a]

Seed treatment	Percentage stand when condition of seed was as indicated: Sound	Cracked	Chipped	Original sample	Mean	Group [b]
None	98.6	90.6	89.3	94.5	93.9	a
Pyrenone	98.0	93.5	90.3	93.5	94.2	a
Ceresan M	88.1	84.0	57.1	80.5	78.4	b
Pyrenone + Ceresan M	86.3	83.2	70.9	80.5	80.5	b
Mean	94.0	88.2	78.4	88.1		
Group [b]	a	b	c	b		

[a] Average of 6 replicates of single-row plots measuring 1 × 10 ft.

[b] Means grouped according to the method of Duncan (5). Treatments having a letter in common are not significantly different from each other; conversely any treatments that do not have a letter in common are significantly different from each other.

Germination in soil.—In the above experiments, no attempt was made to distinguish normal from mercury-injured seedlings. The stubby, mercury-injured seedlings sometimes grew into apparently normal plants. Since the chipped seeds, comprising one third of the total sample, were most susceptible to mercury injury, it seemed advisable to determine the ability of seeds from the various treatments to produce plants. It was thought that the stubby, retarded plants should, perhaps, be more subject to attack by soil fungi than are normal plants or else that they might not become established in time to become anchored before the heaving effects of freezing and thawing begin. Therefore,

TABLE 4.—*Mean height on April 28, 1956, of wheat grown from sound, cracked, and chipped seed treated with combinations of Pyrenone and Ceresan M* [a]

Seed treatment	Mean heights of plants grown from seed in condition indicated: Sound	Cracked	Chipped	Original sample	Mean	Group [b]
	in.	in.	in.	in.	in.	
None	11.7	9.8	10.0	10.7	10.5	a
Pyrenone	11.3	10.7	9.7	10.3	10.5	a
Ceresan M	10.3	9.0	6.0	9.0	8.6	b
Pyrenone + Ceresan M	9.0	9.0	6.3	8.3	8.2	b
Mean	10.6	9.6	8.0	9.6		
Group [b]	a	ab	bc	ab		

[a] Average of 6 replicates of single-row plots measuring 1 × 10 ft.

[b] For an explanation of the statistical groups, see Table 3.

TABLE 5.—*Mean yield of Vahart wheat from sound, cracked, and chipped seed treated with combinations of Pyrenone and Ceresan M* [a]

Seed treatment	Mean yield in bushels per acre when condition of seed was as indicated					
	Sound	Cracked	Chipped	Original sample	Mean	Group [b]
None	47.3	38.8	37.1	41.0	41.0	a
Pyrenone	43.6	40.3	35.3	39.5	39.7	a
Ceresan M	45.9	39.4	19.6	37.7	35.6	b
Pyrenone + Ceresan M	35.7	38.9	24.8	36.0	34.0	b
Mean	43.1	39.3	29.2	38.7		
Group [b]	a	a	b	a		

[a] Average of 6 replicates of single-row plots measuring 1 × 10 ft.
[b] For an explanation of the statistical groups, see Table 3.

germination in soil was deemed necessary.

In December, 1954, 4 months after the seed were treated, bulk samples of the 4 treated lots were planted in greenhouse flats of unsterilized field soil, in a randomized complete block design with 6 replicates. Stand counts were taken after 15 days. Percentage germination was 92.8 for untreated seed, 93.7 for seed treated with Pyrenone, 89.5 for seed treated with Ceresan M, and 89.8 for seed treated with Ceresan M plus Pyrenone. The differences were not statistically significant. These results indicate that seeds from all treatments should produce adequate stands.

Effects of seed treatment on stand and on yield of sound, cracked, and chipped seed.—Separations of seed into sound, cracked, and chipped lots were made from 4 treatment samples of Vahart wheat. An original sample from each treatment was taken as a fourth group. The wheat was planted in 1-row plots in mid-October, 1955, 14 months after treatment, in 6 replicates of a split-plot design, the 4 seed treatments being the main plots and the 4 seed types the subplots. It was realized that in such plots differences due to differences in stands might be accentuated by competition from adjacent rows, but it was thought that the complete randomization within blocks might tend to reduce competition effects. Spring stands and height were estimated on April 28, 1956. Weather conditions were favorable throughout the season, and the plots were in excellent condition at harvest. The entire 1 × 10-ft. plot was harvested. Although the samples were stored for approximately 14 months after treatment, even the plants grown from chipped seeds produced good yields. The yield test should have been planted in the same year that the seed were treated, but the samples were received too late to make the separations.

Highly significant differences in stand, height, and yield were found for treatments and condition of seed (Table 3, 4, 5). There was no statistically significant interaction of treatments × conditions.

The means in Table 5 show that sound seeds outyielded cracked seeds, that chipped seeds yielded the least, and that Ceresan M, but not Pyrenone, affected yield adversely. The treatment means in Tables 3–5 indicate that mercury treatment caused some injury even to the sound seed. It is probable that some of these "sound" seed were cracked but that the fissures were not noticed when the seed were separated. Although statistical determinations were not made, stand, height, and yield appear to be correlated.

DISCUSSION.—The merits of proper use of seed-treatment fungicides have been demonstrated many times. That mercurial fungicides are phytotoxic in excessive amounts or to seed of high moisture content has been accepted and observed by many experimenters. As indicated in the accompanying review of literature, wheat kernels that are damaged in such a way as to expose the embryo are highly susceptible to damage by fungicides, especially as the storage period following treatment is lengthened. Despite the existing reports, the results of numerous studies on the effects of fungicides on seed germination, stands, yields, and varieties of wheat have been published without any apparent regard having been given to the physical condition of the seed used. It should be apparent from the experiments reported here that the proportion of sound, cracked, and chipped seeds should be ascertained for each seed lot used in wheat seed-treatment experiments. Otherwise, the experimenter is apt to conclude erroneously that the seed of 1 variety is more susceptible to fungicidal injury than that of another when, in fact, the seed may have been predisposed differently to injury by improperly operated threshing machines. Such a conclusion might be reversed or not substantiated if sound seed were obtained for subsequent experiments. Hurd (8) stated that the damage that would be done to seed wheat by copper sulphate treatment for smut and by saprophytic fungi could be predicted by examination of the physical condition of the seed. This statement was reiterated by Jones et al (9) and is reiterated here. The mere fact that there has been a shift from copper to mercury compounds has not altered the situation.

Hurd (8) and Zundel (22) have pointed out that damage to the seed may be reduced by slowing the cylinder speed of the thresher. In addition, machines should be properly adjusted. Seedsmen should emphasize these points to their growers. Since it has been shown that prolonged storage increases mercury injury, seed treatment of damaged seed lots should be delayed as long as feasible or the dosage should be reduced. If economy demands that the seed be cleaned, treated, and bagged in 1 continuous operation followed by a prolonged storage period, it is suggested that only those lots containing few damaged kernels be treated with mercury compounds. If spores of bunt fungi are present, however, treatment with mercury compounds is mandatory.

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INVASION AND INFECTION OF CROPS OTHER THAN THE FORMA SUSCEPT BY FUSARIUM OXYSPORUM F. BATATAS AND OTHER FORMAE[1]

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SUMMARY

In greenhouse sand-culture tests, *Fusarium oxysporum* f. *batatas* invaded the colonized roots and stems of inoculated tomato, sweetpotato, cabbage, tobacco, soybean, snapbean, Irish potato, watermelon, cowpea, corn, and cotton plants. The organism caused external symptoms only in sweetpotato. *F. oxysporum* formae *niveum, phaseoli, vasinfectum lycopersici, conglutinans,* and *nicotianae* invaded and colonized roots and stems of inoculated sweetpotato plants but only the last caused obvious symptoms. Cultures isolated from sweetpotatoes inoculated with *F. oxysporum* f. *lycopersici* were less pathogenic to tomato than were the original cultures.

Fusarium oxysporum was isolated from roots and stems of tomato, sweetpotato, cabbage, tobacco, soybean, snapbean, watermelon, cowpea, corn, and cotton planted in naturally infested field soil at 2 locations; however, typical disease symptoms were observed only in sweetpotato plants. When tested, 147 of the 402 isolates obtained from these crop plants were pathogenic to sweetpotato and considered to be f. *batatas.* When compared as groups, isolates from tobacco, soybean, and corn were more pathogenic to sweetpotato than were those from cowpea, snapbean, or tomato, but less pathogenic than were isolates from sweetpotato. Some isolates from tobacco, soybean, and corn, however, were as pathogenic as were isolates from sweetpotato.

On the basis of these findings, it is suggested that the persistence of *F. oxsporum* f. *batatas* and other formae in the soil results from the invasion and colonization of plants other than the formae susceptes in addition to their saprophytic and pathogenic habits.

Fusarium wilt of sweetpotato (*Ipomoea batatas* (L.) Lam.), caused by *Fusarium oxysporum* f.*batatas* (Wr.) Snyd. & Hans., is an important field disease on many farms in the Piedmont and Coastal Plain areas of North Carolina. The disease results in poor stands, low yields, and poor-quality roots. Like other wilt Fusaria, the fungus has been observed to persist in the soil many years in the absence of sweetpotato culture (10). Armstrong and Armstrong (3) have shown that *F. oxysporum* f. *batatas* invades and colonizes the roots and stems of inoculated cotton, Cassia, Mexican clover, okra, sage, soybean, and tomato plants growing in sand culture. In similar tests, sweetpotato roots and stems were invaded by *F. oxysporum* f. *vasinfectum* (Atk.) Snyd. & Hans. and *F. oxysporum* f. *lycopersici* (Sacc.) Snyd. & Hans. Cotton plants grown in fields infested with *F. oxysporum* f. *batatas* were invaded by this forma, although no internal or external symptoms developed. Waite and Dunlap (9) isolated *F. oxysporum* f. *cubense* (E. F. Sm.) Snyd. & Hans. from stolons and roots of Camalote grass (*Paspalum fasciculatum* Willd.), Para grass (*Panicum purpurascens* Raddix), and *Ixophoxus unisetus* (Presl.) Schlecht., and from the roots of *Commelina diffusa* Burm. growing in infested banana plantations.

Several investigators have shown that a wilt Fusarium from 1 crop is pathogenic to plants of other crops. Harter and Field (7) inoculated 9 species of plants with the sweetpotato wilt Fusarium, and it was pathogenic to ivy-leaved morning glory (*Ipomoea hederacea* Jacq.) in addition to sweetpotato. In greenhouse tests, Smith and Shaw (8) found isolates of *F. oxysporum* f. *nicotianae* (J. Johnson) Snyd. & Hans. that were pathogenic to both sweetpotato and cotton. *F. vasinfectum* Atk. from cotton was pathogenic to burley tobacco and cotton. *F. oxysporum* f. *batatas* from sweetpotato was pathogenic to sweetpotato and burley tobacco, and some isolates were pathogenic to flue-cured tobacco. Armstrong et al (5) presented evidence that 1 fungus is involved in causing Fusarium wilt of cotton, okra, Cassia, and tobacco.

The objectives of this research were to determine whether a virulent isolate of *F. oxysporum* f. *batatas* is obviously pathogenic to crops commonly grown in rotation with sweetpotato in North Carolina or whether it merely invades and colonizes the roots of these plants without inciting external symptoms. Similarly, do other formae present in North Carolina damage sweetpotato, or do they only invade and colonize the roots of this crop?

MATERIALS AND METHODS.—The crops and Fusarium formae used were as follows: Porto Rico sweetpotato; Brown Crowder sugar cowpea, *Vigna sinensis* Endl.; New Hampshire Midget watermelon, *Citrullus vulgaris* Schrad.; Kentucky Wonder bunch snapbean, *Phaseolus vulgaris* L.; Trucker's Favorite corn, *Zea mays* L.; Stoneville cotton, *Gossypium hirsutum* L.; Ogden soybean, *Glycine max* (L.) Merr.; Bonny Best tomato, *Lycopersicon esculentum* Mill.; 402 tobacco, *Nicotiana tabacum* L.; Copenhagen Marketer cabbage, *Brassica oleracea* var. *capitata* L.; and Irish Cobbler potato, *Solanum tuberosum* L. The formae of *Fusarium oxysporum* were *batatas, niveum* (E. F. Sm.) Snyd. & Hans., *phaseoli* Kendr. & Snyd., *vasinfectum, lycopersici, conglutinans* (Wr.) Snyd. & Hans, and *nicotianae.* Single-spore cultures were prepared of all formae by the Hansen and Smith technique (6), and pathogenicity of the single-spored lines was demon-

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TABLE 1.—*Incidence of vascular browning in, and frequency of recovery of Fusaria from, various crops grown in sand culture and root inoculated with Fusarium oxysporum f. batatas*

Crop	No. of living plants with vascular browning[a]	No. of plants yielding Fusaria[b]
Cowpea	3	20
Watermelon	0	24
Snap bean	19	26
Cotton	10	23
Corn	0	30
Tomato	0	26
Irish potato	7	15
Tobacco	13	30
Soybean	15	16
Cabbage	15	15

[a] A total of 30 plants inoculated.

[b] Four snap bean, 3 soybean, and 5 cabbage plants died in 1 of 3 tests. Isolations were not made from dead plants. Of the isolates selected from each crop, all were pathogenic to sweetpotato.

strated. Stock cultures of pathogenic isolates were maintained on potato-dextrose-agar slants under mineral oil at room temperature.

In addition to potato-dextrose-agar (PDA) medium used for maintaining stock cultures and for comparing colony characteristics of Fusaria isolated from crop plants, a 20 per cent V-8 (Campbell's V-8 juice, a blend of vegetable juices) agar medium containing 30 ppm each of streptomycin sulfate and oxytetracycline was used for isolation from tissue. All inoculum was grown in modified Richard's solution in shake cultures for 72 hours at room temperature. The solution contained NH_4NO_3 (10 g); KH_2PO_4 (5 g); $MgSO_4 \cdot 7H_2O$ (2.5 g); $FeCl_3 \cdot 6H_2O$ (0.02 g); sucrose (50 g), and water (1000 ml).

Plants of the several crops were grown in sand or soil in the greenhouse and in field soils naturally infested with *F. oxysporum* f. *batatas*. In the sand-culture studies, 10 transplants or seed of each crop were planted around the periphery of 9.5-in. glazed crocks and irrigated daily with nutrient solution (2) until solution drained from the bottom of the crock. Plants requiring mechanical support were tied to sterilized reeds. During winter months, a 15-hour photoperiod was provided with fluorescent lights. Between tests, the crocks were steamed for a minimum of 3 hours. In other greenhouse tests, inoculated plants were grown in 4-in. pots of sterilized soil. The 2 fields infested with the sweetpotato wilt Fusarium were located near Bethel and Benson, North Carolina. The several crops were replicated 4 times in 25-ft. plots.

Isolations were made within 24 hours after plants were removed from sand cultures or field soil. The plant parts were disinfested for 10 minutes in aqueous commercial Clorox (5 per cent sodium hypochlorite) solution diluted 1:10 (3), transferred to sterile Petri dish halves, and sectioned with sterilized razor blades. Four cross sections were cut from each plant, i.e. near the end of the tap root, midregion of the tap root, at the crown or soil line, and 1–2 in. above the soil line. Sections from corn plants were cut from the first 3 internodes. Root and stem cross sections were approximately $\frac{1}{8}$ in. thick, and all sections from a single plant were placed equidistant around the periphery of a Petri dish containing about 25 ml of medium. After a growth period of 24–36 hours at 24°C, mycelial transfers were made from Fusaria colonies to test tube slants of PDA. Plates containing sections from which Fusaria had not grown after 36 hours were incubated for an additional 72 hours before being re-examined and transferred or discarded. Isolates were stored at room temperature until compared with the forma being tested for pathogenicity. The morphological and cultural characteristics of Fusaria from plants of each crop in the field or greenhouse tests were compared with formae stock cultures. Isolates resembling the stock cultures were used in pathogenicity tests.

EXPERIMENTAL RESULTS.—*Inoculation of crops with Fusarium oxysporum f. batatas.*—The several crops growing in sand culture were root inoculated with f. *batatas* by pouring 500 ml of culture fluids containing the fungal growth over injured roots as described by Armstrong (2). One week later all plants were inoculated again and incubated for an additional 6 weeks.

F. oxysporum f. *batatas* was isolated from living plants of all species tested (Table 1). In most cases, Fusaria were obtained from all cross sections irrespective of the portion of stem or root from which the isolation was made. This agrees with Armstrong and Armstrong (3) and indicates that the organism had entered the roots and grown up the stems of the plants. Selected isolates recovered from each crop were pathogenic to sweetpotato. In these experiments, f. *batatas* did not cause external symptoms in any of the crops tested but did cause vascular browning in some of the crops.

Inoculation of sweetpotato with other Fusaria.—Formae of *Fusarium oxysporum* from cotton, tobacco, cabbage, snapbean, watermelon, and tomato were isolated from most of the sweetpotato plants growing

TABLE 2.—*Incidence of symptom development in, and frequency of isolation of Fusaria from, sweetpotato plants grown in sand culture and inoculated with various formae of Fusarium oxysporum*

Formae	No. of dead plants[a]	No. of living plants with vascular browning[b]	No. of living plants yielding Fusaria[b]
lycopersici	1	6/29	29/29
vasinfectum	2	5/28	26/28
conglutinans	1	4/29	28/29
niveum	4	2/26	21/26
phaseoli	0	0/30	28/30
nicotianae	27	2/3	3/3

[a] A total of 30 plants inoculated in 3 tests.

[b] Numerator is number of living plants with vascular browning or yielding Fusaria; denominator is the number of living plants examined. Of the selected isolates of each forma, all were pathogenic to the forma suscept except those isolates from plants inoculated with f. *lycopersici*. They were not pathogenic in 2 tests and only weakly pathogenic in the third.

on sand culture and inoculated as above with the respective formae (Table 2). Only f. *nicotianae* caused obvious symptoms in sweetpotato, which agrees with results reported previously (8). Other formae caused no external vine symptoms, although there were vascular symptoms associated with certain formae in the first test. The representative cultures of formae isolated from sweetpotato, except from plants inoculated with f. *lycopersici*, were pathogenic to the forma suscept in each test. In 2 tests, cultures from plants inoculated with f. *lycopersici* were not pathogenic to tomato; in the third, they were only weakly pathogenic. These results indicate that sweetpotato is a suscept to f. *nicotianae* and can be invaded and colonized by other formae without developing vine symptoms. Of the several formae, *F. oxysporum* f. *lycopersici* was the least successful in invading sweetpotato roots and stems.

Growth of test crops in infested field soil.—The test crops were planted about mid-June in the 2 fields naturally infested with f. *batatas*. Two and 6 weeks later, plants of each crop were examined for external symptoms and 5 plants from each plot were removed for the making of isolations.

For the 2 sampling dates, 40 plants of each crop were examined; external symptoms developed only on sweetpotato. Vascular browning was present in 6 tobacco, 4 cabbage, 3 cowpea, 1 watermelon, 1 snapbean, and 2 cotton plants.

The proportion of plants yielding Fusaria varied with the crop and location (Table 3). At Benson, all crops except soybean and cotton yielded more Fusaria than did tobacco, whereas at Bethel more tobacco plants yielded Fusaria than did those of other crops. From all crops, 402 Fusaria cultures were obtained; of these, 266 resembled f. *batatas* when grown on PDA. When these 266 cultures were tested for pathogenicity to sweetpotato, 147 were found to be pathogenic. Nearly one half or more of the selected isolates from each crop were pathogenic to sweetpotato, with the exception of those from watermelon and cowpea grown at Bethel. It is evident that f. *batatas* invades and colonizes several crop plants grown in naturally infested field soils.

A further evaluation of pathogenicity of these isolates was made in a factorial experiment in which tip cuttings of sweetpotato vines were inoculated in the greenhouse with the 8 most pathogenic cultures from each crop in the above study. The vine cuttings (about 8 in. long) were inoculated by immersing the severed ends in shake culture inoculum and then placing them in 4-in. clay pots half filled with steamed soil. Fifty ml of inoculum were poured around the bases of cut stems, and the pots were filled with soil. Each pot of inoculated plants was placed on an inverted clay saucer during incubation and disease development. Noninoculated cuttings and cuttings inoculated with the stock culture of f. *batatas* were included as controls. Each replication consisted of inoculating 5 cuttings with each isolate; the inoculation was repeated 3 times for all cultures. The number of plants killed and vascular browning were recorded 28 days after inoculation (Table 4).

The Fusaria from the various crops were not equally pathogenic to sweetpotato. Isolates from sweetpotato were almost as pathogenic as were the stock cultures of f. *batatas*. Isolates from tobacco, soybean, or corn were more pathogenic than were those from cowpea, tomato, or snapbean. Whereas the groups of Fusaria from other crops were not as pathogenic as were those from sweetpotato, individual isolates within some groups were equally virulent (Table 4, column 3). Three of the 120 noninoculated plants died; this loss was attributed to the use of some vine cuttings from

TABLE 3.—*Percentage of different crop plants yielding Fusaria when grown in soil naturally infested with Fusarium oxysporum f. batatas, and the percentage of selected isolates pathogenic to sweetpotato*

Crop[a]	No. of plants yielding Fusaria[b]		Percentage of plants yielding Fusaria		No. of isolates tested for pathogenicity to sweetpotato		Percentage of selected isolates pathogenic to sweetpotato	
	Benson field	Bethel field	Benson field	Bethel field	Benson field	Bethel field	Benson field	Bethel field
Tomato	22	12	58.1	33.1	15	8	60.0	50.0
Sweetpotato	26	26	67.5	67.5	12	13	83.3	84.6
Cabbage	15	22	48.4	62.5	13	17	53.8	64.7
Tobacco	9	32	20.0	84.4	7	18	42.9	44.4
Soybean	17	22	44.4	55.0	13	14	53.8	42.9
Snapbean[c]		29		74.4		19		47.4
Watermelon	26	20	78.8	50.0	18	19	66.7	36.8
Cowpea	20	20	52.5	54.4	18	15	61.1	20.0
Corn	24	24	60.0	68.8	11	14	72.7	57.1
Cotton	18	18	44.4	46.9	12	10	66.7	50.0
Total	177	225			119	147		
LSD 5%			27.2	25.7			NS	NS

[a] Irish potato failed at both locations.
[b] Isolations made from a total of 40 plants of each crop.
[c] Snapbean did not emerge at Benson.

TABLE 4.—*Mean pathogenicity to sweetpotato of 8 Fusaria isolated from each of 9 crops grown in field soil naturally infested with Fusarium oxysporum f. batatas*

Source of *F. oxysporum* f. *batatas*	Mean no. of dead plants[a]	Vascular browning index[b]	No. isolates killing all plants
Standard culture	4.8	5.0	
Sweetpotato	4.0	4.5	7
Tobacco	2.7	3.8	3
Soybean	2.5	3.8	2
Corn	2.0	3.4	2
Cotton	1.8	3.3	0
Cabbage	1.6	3.5	0
Watermelon	1.5	3.4	0
Cowpea	1.4	3.1	1
Tomato	1.0	3.0	0
Snapbean	0.8	2.7	0
Noninoculated check[c]	0.02	0.3	
LSD 1%	0.82	0.48	
5%	0.62	0.36	

[a] Mean number of dead plants in 3 replications of 5 plants 28 days after inoculation.

[b] 0 = no vascular browning; 1 = ¼ of the xylem ring discolored; 2 = ½ of the xylem ring discolored; 3 = ¾ of the xylem ring discolored; 4 = 9/10 of the xylem ring discolored; 5 = all of xylem ring discolored or plant dead.

[c] Data not included in the analysis.

roots grown in infested fields.

To eliminate the possible influence of culture metabolites (11) on symptom development and plant death, the 2 most pathogenic cultures from each crop in the above experiment were grown in shake culture and the mycelium filtered out and washed with 100 ml of tap water. The mycelial mats were resuspended in 150 ml of sterile water and used to inoculate sweetpotato vine-cuttings. Plant death and vascular browning symptoms were recorded after 28 days. The removal of metabolic products from the inoculum did not appreciably alter the virulence of the several Fusaria.

DISCUSSION.—The isolation of *F. oxysporum* f. *batatas* from roots and stems of the several crops plants devoid of external symptoms, in both the greenhouse and field tests, is evidence that the fungus invades and colonizes plants other than sweetpotato. This invasion and colonization of various plant roots affords a means for the survival of virulent fungus strains in infested soils for many years in the absence of sweetpotato culture. Although no data were obtained concerning the effect of this phenomenon on persistence of virulent strains of *F. oxysporum* f. *batatas* in field soils over a period of years, the value of crop rotation as a control practice in reducing losses from Fusarium wilt of sweetpotato is open to question.

Fusaria formae inoculated to sweetpotato plants were isolated from a large proportion of the plants. Although this crop was not planted in field soils infested with the several formae, the evidence suggests that sweetpotatoes also serve to perpetuate other Fusaria formae in infested soils, as pointed out by Armstrong and Armstrong (3).

The failure to isolate consistently virulent strains of *F. oxysporum* f. *lycopersici* from inoculated sweetpotatoes does not fully corroborate results reported by Armstrong and Armstrong (3). In a subsequent report (4), they presented evidence of the existence of biological races in the soybean and cowpea wilt Fusaria. Biological races also exist in *F. oxysporum* f. *lycopersici* (1), which could account for this discrepancy between the 2 investigations with this forma.

No attempt was made to determine which root and stem tissues were invaded by the formae inoculated to sweet potato or of other crop plants invaded by *F. oxysporum* f. *batatas*. On the basis of results reported (3) on the adequacy of hypochlorite disinfestation for destroying Fusaria borne superficially, it was assumed that the Fusaria isolated from roots and stems were established within these organs. Armstrong and Armstrong (3) isolated the sweetpotato wilt Fusarium from the wood in the tap root and from the wood and bark of the lower stem of inoculated cotton. Waite and Dunlap (9) observed mycelium of the banana wilt Fusarium in the outer cortex of Para grass stolons.

The invasion and colonization of plant roots by Fusaria formae present an interesting suscept-pathogen relationship. With the exception of *F. oxysporum* f. *nicotianae*, none of the formae incited external symptoms in sweetpotatoes, nor did *F. oxysporum* f. *batatas* incite external symptoms in the plants of other crops. Vascular browning, a typical internal wilt symptom, was present in a number of forma-host combinations, but in other combinations this symptom was absent. The vascular browning symptom suggests that some formae were causing deleterious physiological changes within the suscepts. Measurements of plant growth following inoculation might have revealed a chronic disease reaction devoid of external symptoms; however, the vigor with which most plants grew after inoculation suggested little injury from the invading forma.

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RUST-TMV CROSS-PROTECTION AND NECROTIC-RING REACTION IN BEAN [1]

Eugene M. Wilson [2]

SUMMARY

Cross-protection between bean rust fungus, *Uromyces phaseoli typica* (Pers.) Wint., and tobacco mosaic virus (TMV) was demonstrated in bean leaf tissue. TMV-infected bean leaves showed a resistance to rust (apparently due to inhibition of uredospore germination); rusted bean leaves showed a resistance to TMV infection.

Aqueous diffusates from uredospores, sprayed on bean leaves, protected against both rust infection and the formation of TMV local lesions in the leaves. Diffusate concentration was obtained sufficient to give 81 and 68 per cent control, respectively, of rust pustules and TMV local lesions on Pinto bean leaves. These diffusates inhibited the germination of uredospores.

Aqueous diffusates from bean leaves infected with TMV, sprayed on bean leaves, protected against rust infection. Diffusate concentration was obtained sufficient to give 72 per cent reduction in the number of rust pustules on Pinto bean leaves. These diffusates gave protection against TMV local lesions, but so variable that the results are not considered significant. In rusted and TMV-infected bean leaf tissues, acquired self-protection was reduced by washing the infected tissue with water.

A rust-TMV necrotic-ring reaction (necrotic tissue at the periphery of rust pustules) occurred in bean leaves when rust inoculation preceded TMV inoculation, but not when inoculations were in reverse order. At greenhouse temperatures over 30° C, intervals between inoculations of 1 to 3 days produced much smaller necrotic rings than did intervals of 6 to 8 days. No such effect was observed at lower temperatures. The more mature the rust pustule at TMV inoculation, the more quickly did necrotic-ring formation follow.

Mechanical inoculation of TMV produced only a few small local lesions in Bountiful bean leaves, and no local lesions in bean varieties U. S. No. 3, Harter No. 780, and Harter No. 814. However, rusted leaves of all 4 varieties, as shown by the number of rust-TMV necrotic-ring reactions, were susceptible to TMV infection in a narrow band of tissue at the periphery of the rust infection.

Infection of bean (*Phaseolus vulgaris* L.) leaves with tobacco mosaic virus (TMV) or rust, *Uromyces phaseoli typica* (Pers.) Wint., results in resistance of adjacent healthy tissue to infection with the respective virus or rust (3, 4). Yarwood (3) suggested that this "acquired immunity" may be due to the formation of antibodies. His original evidence gave some indication that the rust resistance was due to a volatile toxic gas liberated by the rust-infected tissue; more recently he reported evidence that water-soluble nonvolatile materials are associated with the phenomenon (4, 6).

The present paper reports experiments in which rust-TMV cross-protection was observed in bean leaf tissue.

Materials and methods.—Bean rust (race 5) and a concentrated solution of tobacco mosaic virus (TMV) obtained by ultracentrifugation from TMV-infected Turkish tobacco plants were used throughout this study. Primary leaves of Pinto bean plants at or near the 10-day stage of growth were used except where otherwise indicated. Inoculations were on the upper surface of the experimental leaf. The virus inoculations were made according to the methods described by Yarwood (2), using 1.0 per cent K_2HPO_4 and carborundum. Rust inoculations were made by atomizing an aqueous suspension of uredospores on the leaves of the experimental plants, which were then held 10 to 12 hours at 16° to 18° C at high humidity in the dark. All *in vivo* experiments were conducted in the greenhouse with plants watered at the soil level to avoid wetting the leaves.

Results.—*TMV resistance from rust infection.*—Leaves infected with rust were inoculated with TMV after varied intervals from the time of rust inoculation. The number of TMV local lesions that developed from these inoculations was progressively smaller as the interval increased, apparently correlated with the maturity of the rust pustules. In some leaves held at 30° C no typical TMV local lesions resulted from inoculations made 6 days after rust inoculation. However, the number of rust-virus necrotic-ring reactions increased with increasing maturity of the rust pustules, as described below.

Aqueous diffusates from uredospores of this rust, sprayed onto healthy bean leaves, protected against TMV local lesion formation. For a dosage-response study of these diffusates, 2 g of uredospores were suspended in 1 liter of distilled water and distributed equally into 6 open halves of Petri dishes (15 cm in

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diameter). Four filter-paper (Whatman No. 1) strips (1 by 6 in., creased 1 in. from the top for hanging on the rim of a Petri dish, and pointed at the other end) were used as wicks in each dish. The diffusates accumulated at the pointed tips of these papers for about 1½ days, at which time the spores in the dish were dry. Then the tip of the dry wick was cut off at about 1 in. from the point. The total of 24 tips were then eluted with 50 ml of distilled water to give a base concentration of uredospore diffusate. This was further diluted with distilled water so as to give a series of concentrations for the dosage-response study. These procedures were carried out at room temperature.

Healthy Pinto bean leaves sprayed with different concentrations of uredospore diffusates and allowed to dry, showed a definite dosage-response effect when inoculated with TMV (Fig. 1). The highest concentration gave 68 per cent control of TMV local lesions. The data in Fig. 1 represent an average of results obtained in 2 to 3 replications of the experiment.

Rust resistance from rust infection.—The aqueous diffusates from uredospores were sprayed on bean leaves at different concentrations and allowed to dry. These leaves were then seeded with uredospores of the same rust. Results showed an 81 per cent control of rust pustules at the highest concentration of uredospore diffusate (Fig. 1).

Acquired resistance against reinfection by the same rust was reduced by washing rusted leaves under the tap for 3 minutes and drying them before reinoculation.

Rust resistance from TMV infection.—Leaves of bean plants with localized areas of TMV local lesions at an early stage were inoculated with the bean rust fungus. After a period of incubation and development, abundant rust pustules appeared except on the leaf areas bearing numerous TMV local lesions (Fig. 2). Microscopic observation of uredospores on the TMV-infected leaf areas showed that these spores failed to germinate. The number and size of rust pustules were not perceptibly reduced on leaves that received TMV inoculation one or more days after rust inoculation.

Inhibition of uredospore germination by TMV-infected leaf tissue was demonstrated by placing such tissue between 2 thin sheets of 2 per cent water agar in Petri dishes and seeding the exposed agar surface with uredospores of the rust fungus. Spores directly over TMV-infected tissue failed to germinate; those over healthy tissue germinated normally. Germ tubes of the uredospores of this rust were not inhibited from germination by a 1.0 per cent K_2HPO_4 suspension of TMV on 2 per cent water agar. The acquired resistance to rust in TMV-infected tissue was reduced by washing the diseased leaves under the tap for 3 minutes and drying them before rust inoculation.

A base concentration of diffusates from TMV-infected tissue was obtained by soaking 6 heavily infected bean leaves (over 300 TMV local lesions per leaf) in 100 ml distilled water for about 16 hours at room temperature. This solution was diluted with distilled water so as to give a series of concentrations of diffusates for the dosage-response study. Healthy bean leaves were sprayed with these different concentrations of diffusate, allowed to dry, and then inoculated with bean rust. The highest concentration of these diffusates gave 72 per cent control of the bean rust fungus (Fig. 1). Diffusates from healthy bean leaf tissue gave no control.

TMV resistance from TMV infection.—To detect this type of resistance, bean leaves were inoculated with TMV, first in transverse strips and 2 days later over the entire upper surface. Zones relatively free of TMV local lesions developed next to the strips first inoculated. The extent of the zone varied, as did the number of local lesions in it. If the strip-inoculated leaves were washed for 3 minutes under the tap and then dried before the second inoculation, the local lesions from the second inoculation were greater in size and 70 per cent greater in number than on unwashed leaves.

The aqueous diffusates from TMV-infected bean leaves gave variable results against TMV local lesions in healthy bean leaves, making conclusions not justified as to their effect.

Rust-TMV necrotic-ring reaction.—When rusted bean leaf tissue was inoculated with TMV 2 to 14 days after the rust inoculation, a dark necrotic ring appeared in 3 to 5 days at the periphery of some rust pustules. The more mature the rust pustule, the shorter was the time interval between inoculation and

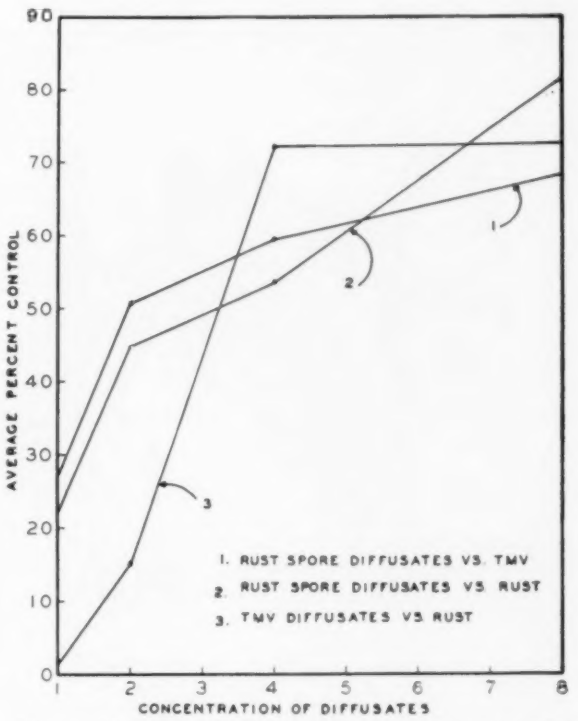
AVERAGE PERCENT CONTROL
CONCENTRATION OF DIFFUSATES
1. RUST SPORE DIFFUSATES VS. TMV
2. RUST SPORE DIFFUSATES VS RUST
3. TMV DIFFUSATES VS RUST

FIG. 1. Control of TMV by diffusates from rust uredospores, and control of rust by diffusates of rust uredospores or TMV-infected bean leaves.

necrotic-ring formation. The tissue within the ring rapidly became necrotic, and further pustule production was limited to very small secondary pustules, at the outer edge of the ring of necrotic tissue.

Temperature influenced the severity of this necrotic response. At greenhouse temperatures over 30° C, intervals of 1 to 3 days between rust and TMV inoculations produced much smaller necrotic rings than did intervals of 6 to 8 days (Fig. 3). This effect was not observed at lower temperatures.

With mechanical inoculation of TMV, Yarwood (1) was unable to infect healthy Bountiful bean plants but was successful with rusted Bountiful bean leaves. This success was based on the rust-TMV necrotic-ring reaction in this host. However, tests by the present author showed that inoculations of TMV in 8- to 10-day-old healthy Bountiful bean plants yielded small, atypical local lesions. When rusted Bountiful bean plants were inoculated with TMV no typical local lesions appeared, but there were a large number of rust-TMV necrotic ring reactions. Only the tissue at the periphery of the rust pustules appeared susceptible to TMV infection. However, the rusted tissue inside each ring became prematurely necrotic. Whether this was due to virus invasion or desiccation of all tissue within each necrotic ring cannot be answered at this time. However, germ tubes of uredospores of this rust fungus were not lysed on water agar, nor were uredospores inhibited from germination by a concentrated suspension of TMV.

Bean varieties U. S. No. 3, Harter No. 780, and Harter No. 814 developed no local lesions when inoculated with TMV. However, if previously infected with rust, they exhibited the same rust-TMV necrotic-ring reaction produced in the Bountiful variety.

Wheat plants infected with *Puccinia graminis* f. sp. *tritici* Erikss. & E. Henn., Race 15-B, did not produce the rust-virus necrotic-ring reaction when inoculated with TMV.

Discussion.—The compounds were not identified in the diffusates from uredospores of the bean rust fungus that caused self-inhibition and protection against TMV infection. Yarwood (4, 1) did not report for bean leaves the rust-TMV cross-protection, though he described the rust-TMV necrotic-ring reaction. Yarwood (4, 5, 6) studied self-inhibition of the bean rust fungus in bean tissue but did not correlate it with any specific chemical. Most workers who have studied the self-inhibition phenomenon exhibited by rust spores agree on two characteristics of the active material: water solubility and volatility at temperatures around 21° C. Yarwood (5), however, found evidence against volatility, as also indicated in the present study. The active materials of the aqueous diffusates of rust uredospores that caused protection in bean against rust and TMV did not evaporate from the leaves, as would be expected of volatile materials.

The present experiments suggest that the substances involved are water soluble. This suggestion may be supported by the fact that resistance to TMV or rust in rusted bean leaves and resistance to TMV in TMV-infected bean leaves are reduced by washing the tissue

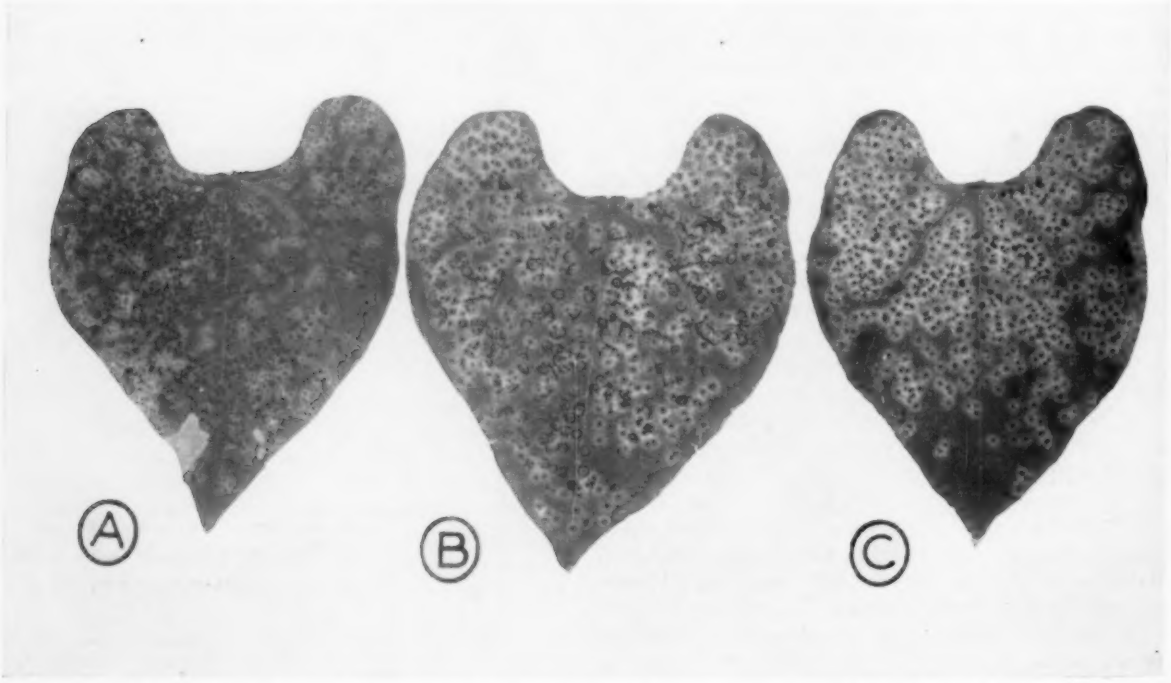

Fig. 2. TMV-induced resistance to bean rust. A) Inoculated in 2 transverse strips with TMV in 1.0 per cent phosphate solution 2 days before the rust inoculation. B) The opposite primary leaf on the same plant as A, treated in the same manner as A except no virus was added to the 1.0 per cent phosphate solution.

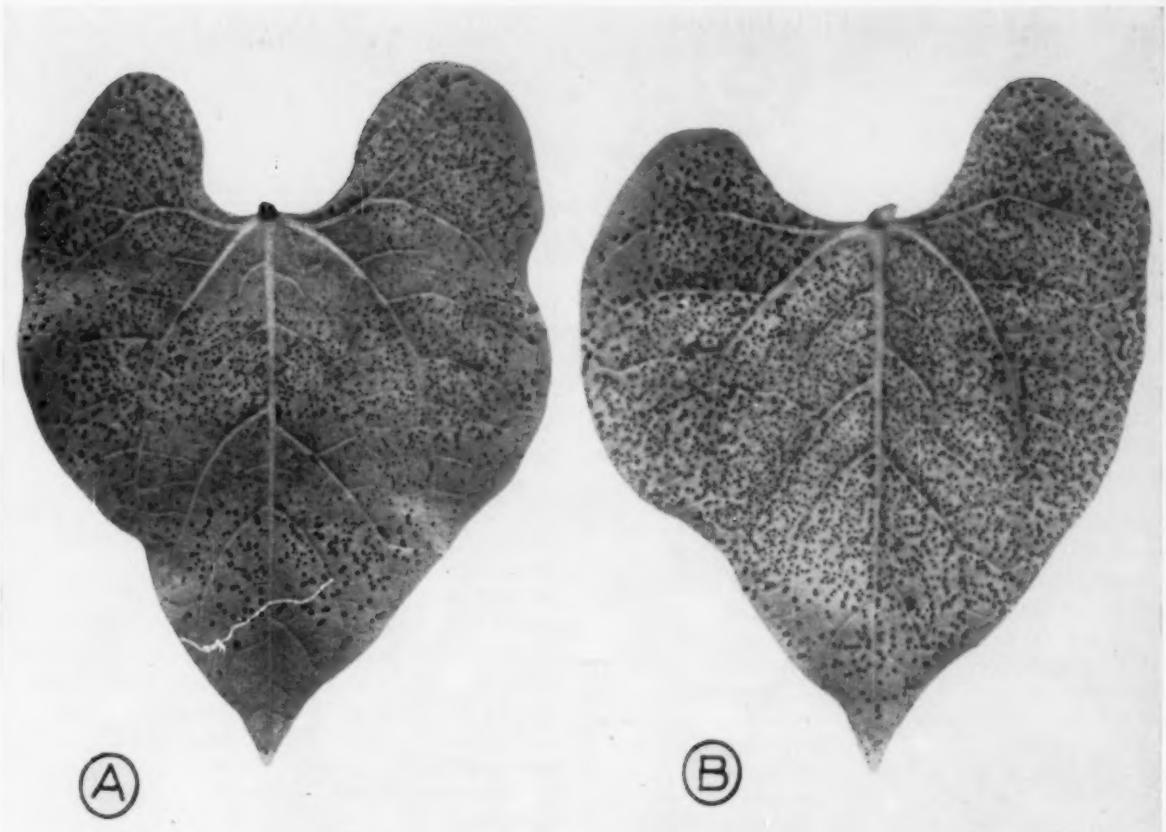

FIG. 3. Rust-TMV necrotic-ring reaction on bean leaves. A) Inoculated with TMV 3 days after inoculation with rust. B) Inoculated with TMV 6 days after inoculation with rust. C) Check, not inoculated with TMV. Note necrotic rings surrounding the rust pustules in A and B, and the larger rings in B, an effect obtained at greenhouse temperatures slightly above 30° C.

for 3 minutes under the tap.

Distribution of the protective substances in the leaves was not studied, but the fact that a brief washing reduces the protection suggests that the substances are on, or close to, the surface of the tissue.

A narrow band of bean leaf tissue at the periphery of mature rust infections is more susceptible to virus infection than adjacent non-rusted tissue in the same leaf, which may become immune to TMV as the rust matures. The most striking example of this greater susceptibility is with the peripheral tissue of mature rust infections on certain bean varieties that are normally immune to TMV infection. Temperatures of over 30° C appear to hasten susceptibility of the rusted tissue to TMV infection. The cause of this increased susceptibility to TMV at the periphery of the rust infection, simultaneous with decreased susceptibility farther from the same rust pustule, has not been determined.

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A ROT OF SMUTTED INFLORESCENCES OF CEREALS BY FUSARIUM POAE IN ASSOCIATION WITH THE MITE SITEROPTES GRAMINUM [1]

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SUMMARY

The mite *Siteroptes graminum* (Reuter), in close association with *Fusarium poae* (Pk.) Wr., developed abundantly in smutted inflorescences of cereals in the greenhouse. In humid summer weather it also occurred in field plots. The affected inflorescences failed to emerge from the sheaths and were eventually destroyed by a wet rot resulting from the combined action of mite and fungus. When female mites, with their bodies distended, were plated on agar, colonies of *F. poae* developed, even after surface-sterilization of the mites. The mite was controlled in the greenhouse by spraying plants weekly with 0.05 per cent solution, commencing when the plants were in the four-leaf stage.

In studies on the genetics of seedling-infecting smuts of oats and barley, the crowding of plants in the greenhouse provided ideal conditions for the multiplication of various parasites and pathogens. The occurrence of a mite, *Siteroptes graminum* (Reuter), in association with *Fusarium poae* (Pk.) Wr. in smutted florets of cereals was first noted in 1956, when several smutted heads of barley (*Ustilago hordei* (Pers.) Lagh. and *U. nigra* Tapke rotted within the sheaths (Fig. 1, B, C, D). These decayed smut heads were permeated by mycelium of a species of *Fusarium*. Embedded in the mycelium and the smut spores were oval, semi-transparent bodies (Fig. 1, A, C). Since the bodies varied considerably in size it was thought that they were not insect eggs. No further effort was made at that time to determine their identity. The incidence of the rot of smutted heads gradually increased until, in plants coming into the boot stage in January to April, 1957, more than 50 per cent of the smutted inflorescences were lost. This loss was serious, for all the smut consisted of F_1 lines derived from paired monosporidial cultures. It was clear that investigations were needed to develop a method of control of this disease.

When a number of these egg-like bodies (Fig. 1, A, C) were plated on potato-sugar agar in Petri plates, each yielded a colony of *F. poae* and a number of mites. Colonies of *F. poae* developed even after mite bodies had been surface-sterilized in mercuric chloride.

The mite was identified by Dr. H. H. J. Nesbitt, Carleton University, Ottawa, Ontario, as *Siteroptes graminum* (Reuter). Before he examined the specimens with the compound microscope he mistook them for the very closely related genus *Pyemotes* (= *Pediculoides*), in which gravid females are large, as in *Siteroptes*.

Hodgkiss[4] reported that a mite found on grass in Finland was described by Reuter in 1900, as *Pediculoides graminum*. The same mite was first found in the United States in 1905, causing a rot of carnation buds. In 1908 Heald and Wolcott described the mite in carnation buds under the name of *Pediculoides dianthophilus*, but by that time Reuter had recognized characters of generic significance in his species and had created the genus *Pediculopsis*, with *graminum* as the type species. Later workers, recognizing that Reuter's species *P. graminum* was the same as that described by Amberling (1861), transferred it to the genus *Siteroptes*.

The same mite was later reported[5, 6] as a pest of grasses and grains as well as the active agent in dissemination of the spores of *Sporotrichum poae* Pk. in the United States. Infested wheat, oats, barley, and rye were malformed and stunted, and their inflorescences were often silvered. Hence the common name "silver top." Hodgkiss[4] described the effects of the mite on grass and on carnations as being quite dissimilar, although in both cases it was associated with the same fungus, *S. poae*. Leach[6] (p. 398-400) described the activities of the mites as follows: "The mites crawl down the grass culms under the sheath to the base of the panicle where the tissues are relatively soft and succulent. They inoculate the tissues with the fungus which they carry with them. The mites apparently feed on the fungus and the decayed tissue that develops at the base of the panicle." Cooper[7] reported experi-

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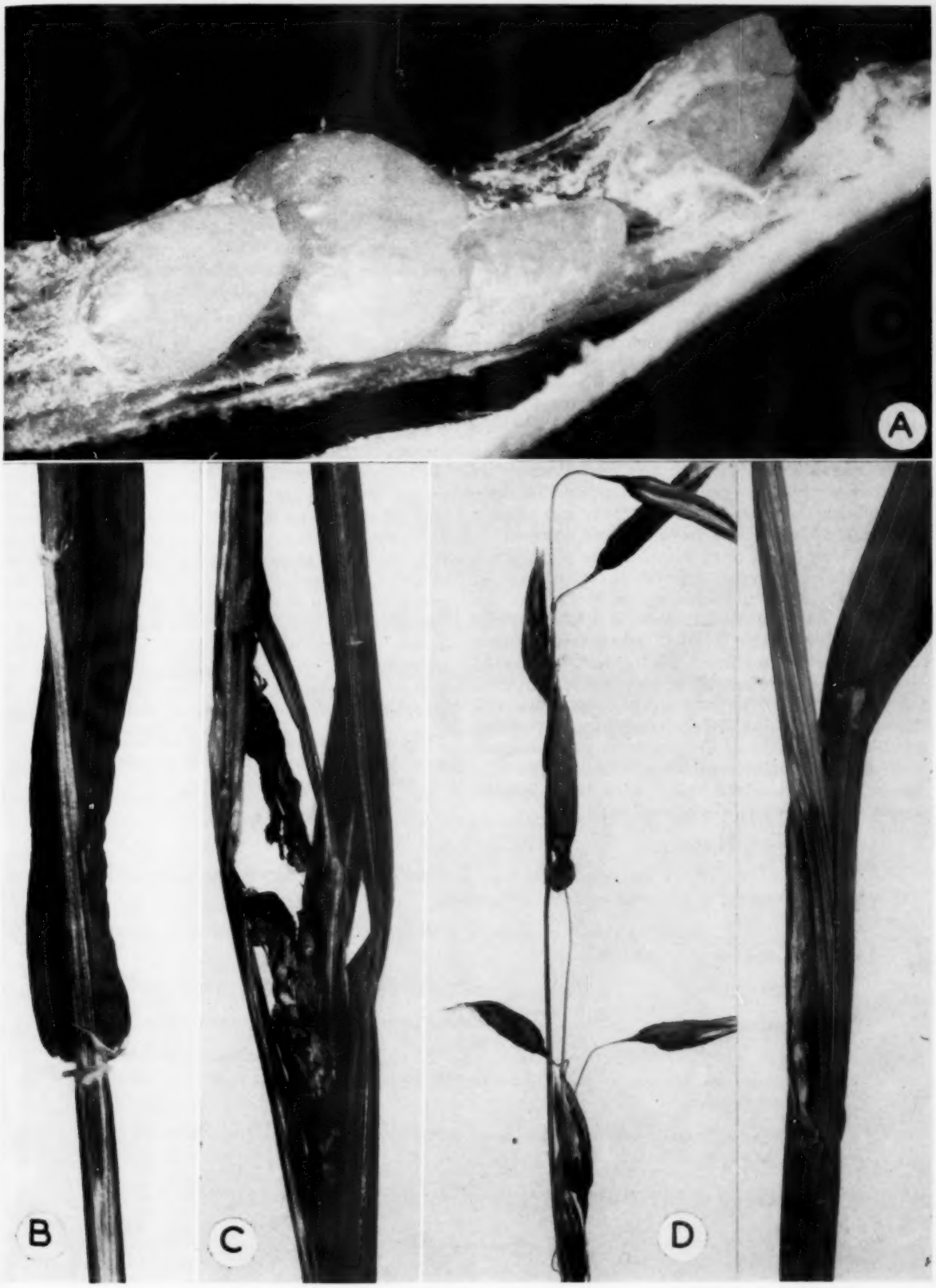
[6] Leach, J. G. 1940. Insect transmission of plant diseases. McGraw-Hill, New York. 615 p.

[7] Cooper, K. W. 1940. Relations of Pediculopsis graminum and Fusarium poae to central bud rot of carnations. Phytopathology 30: 853-859.

FIG. 1. A) Distended hysterostomas of the mite, *Siteroptes graminum*, on rotted barley smut head (× 20). B) Smutted head of barley within the sheath affected by *Fusarium poae* and the mite. C) Same with the sheath torn open. D) Smut of oats—rotten on the right and normal on the left—from a tiller of the same plant after weekly sprays.


A
B
C
D

ments to demonstrate that the mite *P. graminum* introduced *Fusarium poae* into carnation buds. He classed the relationship as a type of "antagonistic symbiosis."

The life history of *S. graminum* under field conditions is apparently not completely known. In the greenhouse it thrives in an active stage the year around. The method of reproduction is extremely unusual. The birth process, as described by Baker and Wharton[5] (p. 165-167), was observed during the present study. While a female is attached to food, its hysterostoma (posterior part of the body) swells to 100 to 500 times the original size (Fig. 1, A), and within this sack the eggs are formed and develop into adult mites. Under magnification the young and adults can be seen through the semi-transparent integument, moving about freely. A mass birth occurs by breakdown of the hysterostoma, though a few immature forms or adults may be extruded before the mass birth. It has been reported[5] that "only a few males are born, each brood containing at least one male. Mating takes place within the sack before birth, as well as after birth."

The mites in Petri plates were seen feeding on Fusarium mycelium. They survived as long as there was sufficient moisture in the agar; in fact, female mites with distended hysterostomas were observed on agar, but they failed to reproduce if the plates dried up. In the greenhouse, enlarged hysterostomas of female mites dried up within a few hours after the sheath over an affected smut head was torn open (Fig. 1, C). In the summer of 1957, when very frequent rains occurred up to July 10, the incidence of decayed smut heads in experimental field plots of wheat, oats, and barley, was over 10 per cent, the highest observed. During the latter part of July, when hot, dry weather prevailed, only dried-up hysterostomas were observed in the field. With the return of moist weather, in early August, numerous viable mites with large hysterostomas were present on the affected smut heads.

The present studies revealed no antagonism between the mite and *F. poae*. The fungus, in addition to providing food for the mites, causes the rotting of smutted heads, thus providing the moist conditions necessary for mite propagation. Moreover, since the affected inflorescences remain enclosed within the sheaths (Fig. 1, B, D), a long period is provided for development of the mites. Mites disseminate the fungus and inoculate the host by wounding the young tissues. It therefore seems to the authors that there exists a true, though not obligate, symbiosis between the two organisms.

During the present studies, neither in the greenhouse nor in the field plots was any sign observed of mite infestation on the healthy inflorescences of cereals. This mite may therefore not be the species reported[1, 4] to cause silver top on cereals and grasses.

Systox (Demeton, B-ethylmercaptoethyl diethyl thionophosphate), a systemic insecticide, seemed to have no effect on mites, even at phytotoxic doses. A few sprayings, 10 days or more apart, with 0.05 per cent Kelthane (1,1-bis (p-chlorophenyl)-2,2,2-trichlorethanol) and endrin also had little apparent effect on the mites within the sheaths. Even dipping the affected plants into 0.05 per cent (2 pints of 20 per cent endrin in 100 gal.) endrin solution did not kill the mites inside the sheaths. However, plants in the fourth- or fifth-leaf stage sprayed weekly with 0.05 per cent endrin were free of mite infestation. Even tillers from the affected plants, which at the commencement of a weekly spray schedule were in fourth-leaf stage, yielded normal smut heads (Fig. 1, D). It seems that it should be possible to eliminate the mites from the greenhouse without destroying all the affected plants.

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